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# JOURNAL OF DAIRY SCIENCE

VOLUME XXI

JANUARY, 1938

NUMBER 1

## A TEST FOR EXTRANEEOUS MATTER IN CHEESE

D. W. SPICER<sup>1</sup> AND WALTER V. PRICE

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The requirements of the Federal Food and Drug Administration have stimulated the interest of the cheese industry in methods of detecting extraneous matter in cheese. Greene<sup>2</sup> has described a method which works well on some types but is somewhat slow in dissolving hard and semi-hard types of cheese. A satisfactory sediment test should make it possible to remove foreign material from American or Brick cheese without altering materially the physical condition and appearance of the extraneous matter which might be in it. Such a test to be of value in practical control work should be effective with cheese two weeks old. Rapidity and ease of performing the test and low cost of materials and equipment are highly desirable. This study was undertaken to attempt the development of a test with these characteristics.

First, it was necessary to determine the influence of the size of cheese particles, the type of filter, speed and type of agitation of the cheese with the solvent, type of solvent, concentration of solvent, temperature and time of exposure to solvent action, and the age of the cheese. Finally, after the most suitable conditions and solvents for cheese dispersion were determined, various materials which might possibly contaminate cheese were subjected to these treatments. Some of the substances exposed to such treatments were flies, cockroaches, human hairs, cow hairs, broom splints, brush hairs, cloth, copper and wood splinters. Many of the solvents were eliminated because of their effect upon foreign material.

The size of the cheese particles was varied from  $\frac{1}{4}$ -inch cubes to the mass resulting from the use of a regular meat grinder. A kitchen type food grinder eventually proved most satisfactory for macerating the cheese. The knife was removed from the grinder so that the cheese was simply forced or crushed through the perforated end plate. Foreign material retained its identity when this method was used.

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<sup>1</sup> Industrial Fellow on a grant of the Kraft-Phenix Cheese Corporation.

<sup>2</sup> Greene, W. S. A method for the detection of extraneous matter in cheese. *Cheese Reporter*. March 21, 1936.

Mixtures of cheese and various solvents were stirred in one quart milk bottles with an ordinary laboratory electric mixer equipped with a bent glass stirring rod. The speed of the mixer could be adjusted but for ordinary use the revolutions per minute approximated 1000. It was desirable to place a guard plate on the stirring rod to protect the open mouth of the milk bottle from oil or dust from the mechanical stirrer.

Several kinds of filter papers, cloths and ordinary milk sediment discs were used for removing sediment from the solutions of cheese. The regular milk sediment discs proved most satisfactory for filtering but not so good for examining the sediment because foreign material tended to become imbedded and hidden in the cotton fibers and because under the microscope the fibers of the pad could be confused with white hairs. These objections were minimized by reversing the filter pads, so that the sediment was left on the compact side of the pad and remained clearly visible.

The filter pads were used in both a vacuum milk sediment tester and a pressure milk sediment tester. The vacuum tester usually left a small amount of the solution on the pad. Before the pad could be removed from the tester this small amount of solution had to be poured or washed off, and frequently this lost some of the sediment. It was also difficult to wash the pad of the vacuum tester with hot water in order to remove undissolved fat. The pressure type of milk sediment tester proved to be most suitable. Light pressure from the laboratory air line instead of the usual rubber bulb was used to force the liquid through the filter. This was very convenient when a large number of samples were tested, but the rubber bulb was just as effective.

It was difficult to find a solvent that would release insoluble material in the cheese without changing the physical characteristics of the foreign material. Various concentrations and combinations of a number of chemicals were tried. Concentrations were varied from about 0.25 per cent to 20 per cent while temperatures of heating were varied from 20° to 100° C. and maintained from 10 minutes to 18 hours. Each solvent seemed to produce its best action on cheese under certain optimum conditions of concentrations, temperature and duration of contact with the cheese. These optimum conditions were determined by repeated trials and then the various solvents were compared. All solvents were criticized for their action on cheese, action on foreign material, speed of solution, ease of filtration, and undesirable residue left on the pad. The following solvents and combinations of solvents dissolved cheese efficiently when used under the proper conditions: Minnesota-Babcock reagent; sodium bicarbonate; sodium hydroxide; di-sodium phosphate; tri-sodium phosphate; hydrochloric acid; sodium citrate; tetra-sodium phosphate; tri-sodium phosphate and di-sodium phosphate; and sodium citrate and hydrochloric acid. Commercial pepsin was also tried but was too slow in action to be practical. It is not necessary here to

discuss the specific effects of all of these substances. Solvents which seemed most desirable in several respects failed to satisfy in some other manner. For example, it was found that Minnesota reagent and concentrated hydrochloric acid discolored the cheese and sediment pad, while solutions of cheese made with sodium hydroxide filtered in a satisfactory manner but tended to decompose foreign material.

The most satisfactory solvent was found to be 150 grams of sodium citrate dissolved and diluted in distilled water to 1000 ml. The use of a small amount of hydrochloric acid with the sodium citrate frequently hastened solution but was undesirable because sometimes the solvent mixture formed a slime which delayed filtration. This sodium citrate solution can be used on the common varieties of cheese. In the course of this study it was used on the edible portions of Swiss, American, Brick, Limburger and Cream cheese. The rind on Limburger, which is sometimes eaten, would not always dissolve in a satisfactory manner with the sodium citrate solution.

The age of the cheese affects the ease of solution. Old cheese disintegrates more easily than fresh cheese. Trials with freshly made Brick and American cheese indicated that prolonging the exposure of the cheese to the solvent accomplished the desired results. Cheese was soaked at 37° C. for 18 hours in this solvent without affecting the common types of extraneous matter which might contaminate it. More than 200 samples of Brick cheese, collected over an eight months' period from 40 different factories, dissolved readily in the sodium citrate solution. In not more than 15 instances was a 30 minutes' exposure to the solvent inadequate.

The details of the procedure which was finally adopted to determine the extraneous matter in the cheese are presented here.

#### PROCEDURE FOR DETECTING EXTRANEOUS MATTER IN CHEESE

The equipment and materials for making the test consist of a meat grinder; torsion balance capable of weighing 100 grams; clean 200 cc. beakers; clean quart milk bottles; parchment paper; cheese knife; adjustable speed, electric stirrer; water bath; sediment tester discs; pressure sediment tester; hot plate or Bunsen burner; and a wash-bottle for distilled water. The solvent solution is made by dissolving 150 grams of sodium citrate in distilled water and then diluting the solution to 1000 ml. The solution is always filtered before use.

The cheese is prepared for analysis by placing it on clean parchment paper, removing the rind with a knife, and cutting the cheese into strips of suitable size to feed into the food chopper. Extreme care must be taken to keep particles of paraffin or dust specks out of the sample. The grinder must be clean and free from rust and sediment. The cutting knife should be removed so that the cheese is only forced through the usual perforated end plate of the food grinder. The crushed cheese is collected in a clean 200

cc. beaker. After each sample is ground, the machine must be cleaned by washing.

Exactly 100 grams of the crushed cheese is weighed into a clean quart milk bottle which has been thoroughly washed and rinsed with distilled or filtered water. Then 200 ml. of the filtered solvent solution is added to the sample of cheese and the milk bottle is placed in the water bath at 61.7° C. (140° F.). At higher temperatures protein material tends to coagulate and produces slime which clogs the filter pad.

The mechanical agitator is adjusted in the bottle and the mixture stirred at approximately 1000 r.p.m. for 15 minutes. A 100 cc. portion of distilled water and 100 cc. of the solvent solution are then added and the mixture is again agitated, this time until the cheese particles are entirely dissolved. Ordinarily not more than 30 minutes will be required to accomplish this dispersion. The contents of the milk bottle are then divided into two equal parts and each part is filtered through a single sediment disc. The sediment discs are reversed in filtering so that the sediment remains on the compact surface. After each filtration the sediment tester must be thoroughly rinsed with distilled or filtered water so that all sediment will be on the pad.

One filter pad will usually accommodate the entire sample but in general it is advisable to divide the cheese solution into two equal parts and then pass each half through separate discs. Since 100 grams of cheese represent about one quart of milk, then each sediment disc resulting from this procedure contains the residue from a pint of milk and can, therefore, be compared with the standards used for judging sediment tests of whole milk.

Interpretation of the results of any sediment test must be somewhat arbitrary. Accepted standards for market milk may be applied when a sample of cheese weighing 50 grams is filtered through a single disc. In this study a low power binocular was used to identify extraneous material in cheese. Examinations of discs under 25X and 75X magnification were made as a matter of routine. It was convenient to classify the results of the tests as good, fair, unsatisfactory and bad. A good disc is practically clean to the eye and is microscopically free from material pertaining to animals and insects. A fair disc to the eye shows the presence of a small amount of sediment, but is microscopically free from material pertaining to animals and insects. An unsatisfactory disc to the eye shows the presence of an appreciable amount of sediment, but is microscopically free from material pertaining to animals and insects. A bad disc contains hairs or other materials pertaining to animals and insects.

#### PRACTICAL APPLICATION

Brick cheese was purchased from about 40 Brick cheese factories at intervals throughout the year, tested for extraneous matter and finally was scored at six weeks of age.

Table 1 shows the relation of sediment in cheese to the quality of the

TABLE 1  
*Relation of sediment to the grade of Brick cheese*

SEDIMENT TEST	NUMBER OF SAMPLES	PER CENT OF SAMPLES	AVERAGE SCORE OF CHEESE
Good .. ...	7	5.88	89.7
Fair .. ...	56	47.06	89.4
Unsatisfactory .. ...	36	30.25	88.4
Bad .. ...	20	16.80	88.1

cheese. As the sediment in the cheese increased, the quality of the cheese decreased. Sediment is not the fundamental cause of poor cheese. Sediment is rather the evidence of improper production and manufacturing methods. Careful farmers and skillful cheese makers in their efforts to control the quality of milk and cheese protect these products from contamination. Their equipment is cleaner and their methods are more desirable than those of less careful producers and operators. Filters could remove extraneous matter without changing the quality of the cheese. Milk and cheese, however, should be clean without the use of filters.

This test has been adopted for use in at least one commercial laboratory, and for a period of more than a year has proved to be a satisfactory method of checking the cleanliness of production methods.



## NATURE'S COMPENSATION FOR THE LOST QUARTER OF A COW'S UDDER

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In following the regular procedure of studying the structure of the cow's udder in relation to its comparative development in early life and its subsequent producing ability, the Bureau of Dairy Industry recently recorded a case that appears to throw some light on the question of whether or not Nature compensates for the loss of one or more quarters of the udder by increasing the activity of the remaining quarters. The case in question is that of cow No. 1074, a registered Jersey bred and raised in the Bureau's herd at the National Agricultural Research Center, Beltsville, Md.

The udder of this animal was examined periodically beginning at the age of 1 month 26 days, according to the program regularly followed with all heifers in the breeding herd. The notes recorded at the different observations up to 11 months 28 days of age include nothing to suggest any deficiency or abnormality in the quantity or form of glandular tissue in the udder. In fact, at each of these observations excepting the first, the mammary gland development appears to have been well above the average. At the observation made at the age of 1 year 5 months 22 days, however, the udder was poorly shaped, and the glandular development was definitely below average for length, width, and depth excepting for the right rear quarter which showed an abnormal enlargement and contained a large, round, non-compressible, hard lump about 2½ inches in diameter. The cause of this condition is not known. Apparently the more or less acute condition described disappeared during the following months as there is nothing in the record of the four observations taken during the two months before calving to indicate any lack of uniformity or any deficiency in the udder.

At the observation made on May 10, 1934, two days after calving, the swelling incidental to parturition was extreme, and the udder was definitely plastic especially in the rear. The term "plastic" is used to mean that localized external pressure leaves a persistent indentation. The notes recorded on that date, however, show that the left rear quarter was "blind," the right rear quarter contained a little fluid, and the left front quarter was below normal in fullness. The left rear quarter declined in size more rapidly than the others, though the udder was not markedly unbalanced, and the "feel" of the front and rear quarters did not differ greatly. Notes recorded

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<sup>1</sup> Resigned May 31, 1937.

on July 12, 1934, show "both rear quarters blind." This cow, therefore, came into milk with the left rear quarter entirely inactive and the right rear quarter yielding only a little fluid, and both rear quarters were "blind" almost from the commencement of lactation. In fact normal milk was never obtained from either rear quarter of the udder.

Production for 365 days during the first lactation period which commenced at 2 years 6 months of age, amounted to 7,223 pounds of milk and 410 pounds of butterfat. The highest month's production amounted to 775 pounds of milk and 42.01 pounds of butterfat. Calving again at 3 years 9 months, and milked three times daily as before, she produced 677.3 pounds of milk and 31.67 pounds of butterfat in the first 20 days before the onset of septicemia resulting from udder infection, which caused her death. Compared with the 509.6 pounds of milk and 21.71 pounds of butterfat produced during the first 20 days of her first lactation, this represents an increase of 32.9 per cent in milk and 45.9 per cent in butterfat. This is a much greater increase than that attributable to age and, although it represents only a limited period of time, it indicates that the first record did not overemphasize the cow's ability to produce.

Figure 1 shows the external appearance of the udder of this cow on



FIG. 1. Cow No. 1074, 9 days after second calving and shortly before the onset of the udder infection that caused her death.

August 1, 1935, nine days after her second calving and only a few days before the onset of the udder infection that caused her death. Figure 2 represents a vertical longitudinal section through the left half of the udder, through the plane of the teats. It is readily discernible that the tissues of the front (usually the smaller) quarter had pushed backward and displaced

a large part of the space normally occupied by the rear quarter. In fact if a vertical transverse cut had been made through the rear quarter it would have passed through tissue more than half of which apparently was contributing to the secretion of the front quarter. Figure 2 shows the surface on the median side of the section, making the rear teat appear at the left side of the picture.

Figure 3 shows the rear surface of a vertical transverse section through the right half of the udder. The surface illustrated represents a plane directly above the center of the rear teat. Although the illustration in Figure 3 is entirely different in appearance from that in Figure 2, owing to the manner of sectioning, it shows that essentially the same change had occurred in both the right and left halves of the udder. In Figure 3, for example, the tissue identified with the rear quarter is confined to a thin, dark-colored layer along the base and a small dark-colored area which appears in the upper left portion of the section. The rest consists of tissue of the front quarter that had crowded toward the rear until it occupied most of the area above the rear teat normally occupied entirely by rear quarter tissue. In this plane, directly above the rear teat, the front quarter appears to occupy about two-thirds of the glandular portion of the section.

The occurrence of production records made when one or more quarters of the udder are non-functioning is a source of no little annoyance to one making a comparative study of the innate producing capacity of dairy cows. This situation is often met in evaluating the transmitting ability of herd sires on the basis of the comparison of the production of the sire's daughters with that of their dams. Sometimes, under such circumstances, attempts have been made to estimate the quantity of milk or butterfat the cow would have produced if all of her quarters had been functioning normally. Some have attempted to compensate for such a handicap by crediting the cow with additional amounts representing certain percentages of the quantity of milk or butterfat she actually produced. Such credits might be based on the assumption that a cow milking from three quarters of the udder would produce only three-fourths as much as she would if all four quarters of the udder were functioning. Even if that theory were sound it would appear that consideration should be given to the question of whether the "blindness" occurred in a front or in a rear quarter as it is fairly well established<sup>2,3</sup> that, on an average, the front quarters produce only slightly more than 40 per cent of the total yield of the udder. But what certainty is there that a "blind" quarter does constitute a serious handicap? Is it not reasonable to believe that if one quarter of the udder fails to function the other quarters may assume greater activity and at least partly compensate for the loss?

<sup>2</sup> Turner, C. W. The functional individuality of the mammary glands of the udder of the dairy cow. Mo. Res. Bull. 211, p. 48. 1934.

<sup>3</sup> Unpublished data of B.D.I.

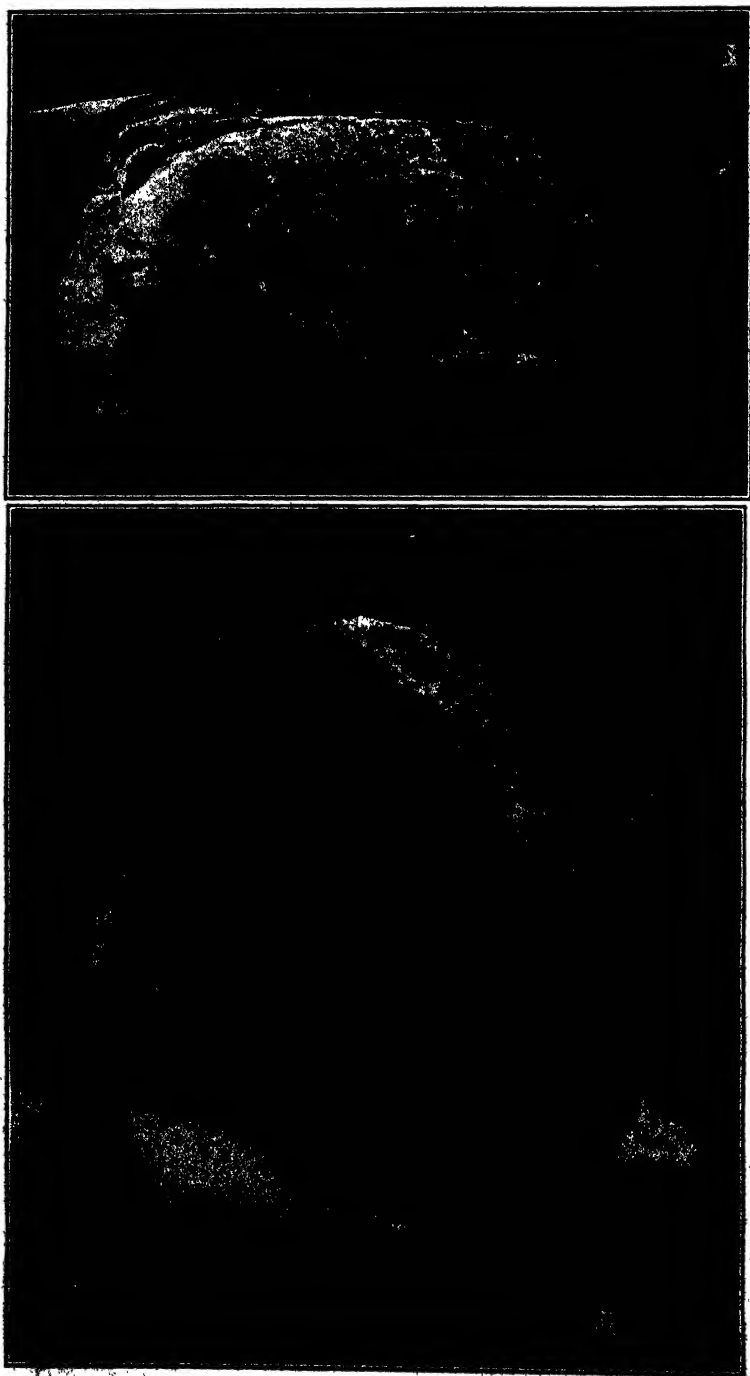


Fig. 2. Vertical longitudinal section through the left half of the udder of cow No. 1074, in the plane of the teats. Photograph shows median surface of section. Dotted line shows extent to which the front-quarter tissue has invaded the rear-quarter territory.

Fig. 3. Vertical transverse section through the right half of the udder of cow No. 1074 in a plane above the front half of the rear teat. Photograph shows posterior surface of section.

If one were to attempt to adjust the two-year-old, 365-day production record of this cow (No. 1074) to compensate for the loss of both rear quarters, and consideration were given to the normal expectancy of approximately 40 per cent of the total yield of the udder being produced by the two front quarters, the calculated producing capacity of this cow would be 18,057 pounds of milk and 1,025 pounds of butterfat as a 2-year-old. Correcting further for age on the basis of accepted factors would give an estimated mature production capacity of 23,294 pounds of milk and 1,282 pounds of butterfat for this cow. There is no reason to believe she possessed an inheritance for production that even approached such a high level.

In view of the record made by this cow in her first lactation, and the position of the front quarter tissue above the rear teat in an udder that did not suffer severely in fullness in the rear as shown by Figures 1 and 2, there can be little doubt of the ability of Nature to compensate to a large extent for the loss of quarters—particularly when such a loss occurs early in life—though there is no certainty that such compensation always occurs.



# AN EVALUATION OF SEVERAL METHODS OF COOLING CREAM<sup>1</sup>

W. J. CAULFIELD AND W. H. MARTIN

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One of the practical difficulties encountered in the section of the central west where a large percentage of the butterfat is marketed as sour cream is to provide adequate refrigeration for the cream prior to churning. The enormous losses from defective butterfat, especially during the hot months of the year, make it imperative that some simple, practical method of cooling cream be devised. Mechanical refrigeration has not been widely used in the production of cream for buttermaking because of the comparatively small volume of cream produced on the average farm. The two most common methods of cooling which have been used in the cream buying stations are the wet sack or evaporation and the spray systems. Although these methods of cooling cream have been used for a number of years, little is known about their actual value and reliability. Fouts and Keith<sup>2</sup> have recommended them for use in Oklahoma, and have given plans and specifications for the construction of a spray cooler. The factors affecting the efficiency of these two systems of cooling have been studied by Martin, Fay and Caulfield,<sup>3</sup> who concluded that neither of these methods of cooling should be regarded as a final and satisfactory method for cooling cream in the station. They recommend their use, however, until some more practical method could be perfected. Numerous other studies have been made of the cream cooling problem; however, they have not dealt with the evaporation or spray method of cooling.

The purpose of this investigation was to determine the relative merits and also the limitations of some of the more commonly used methods of cooling cream including: (1) placing cans of cream in a refrigerator, (2) partially submerging the cans in water, (3) allowing water to flow over the outside of the can, (4) using a spray, and (5) using evaporation. In addition, the effectiveness of the evaporation method of cooling sweet and sour cream through a wide range of atmospheric temperatures and humidities was determined. The feasibility of using the evaporation method to prevent a rise in temperature of cream which had been previously cooled was also studied.

## PROCEDURE

The cream used in this study contained 35 per cent butterfat. Forced air circulation was provided in all trials in which the evaporation method

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<sup>1</sup> Contribution No. 120 from the Department of Dairy Husbandry.

<sup>2</sup> Fouts, E. L., and Keith, J. I. Improving the quality of Oklahoma butter. Okla. Agr. Exp. Sta. Bul. 226. 1935.

<sup>3</sup> Martin, W. H., Caulfield, W. J., and Fay, A. C. The evaporation and spray systems of cooling cream. Kan. Agr. Exp. Cir. 180. 1936.

of cooling was used. In all cooling trials the cream was stirred at thirty minute intervals through the first six hours of each trial. Unless otherwise stated the temperatures of the cream were taken after stirring the contents of the can.

#### COMPARISON OF FIVE DIFFERENT METHODS OF COOLING CREAM

Five ten-gallon cans of sour cream were adjusted to approximately 90° F. and cooled by five different procedures. The trial was then repeated and two similar trials were made using sweet cream. One can was placed in a refrigerator maintained at 35–40° F.; a second can was placed in a cooling tank through which flowed continuously 126 gallons of water per hour, at 66–68° F. (submerged); a stream of water of the same volume and temperature as was used in the cooling tank was directed into the lid of a third can and allowed to flow over the outside of the can (flooded); a fourth can was placed under a coarse spray which delivered 220 gallons of water 67–68° F. per hour, and the remaining can was covered with a wet sack, placed in a room with a temperature 92–98° F. and cooled by evaporation. The rate of temperature change which occurred in each of the five cans during the cooling period was recorded at thirty minute intervals.

Under the conditions of this trial the rate of cooling during the first two hours was fastest by the submerged method, this being followed in order by the flooded, refrigerator, spray and evaporation methods (Fig. 1). At the end of six hours the cream placed in the refrigerator was cooled to a slightly lower temperature (2–4° F.) than that cooled by either the submerged or flooded methods of cooling, and to from 5–12° F. lower than the cream cooled by either the spray or evaporation methods. The use of a spray proved somewhat more efficient than the exaporation method in this comparison.

Sour cream, irrespective of the method of cooling, did not cool so rapidly nor to quite so low a temperature as did the sweet cream (Fig. 1). The greatest difference in the final temperature of the cooled sweet and sour cream, however, amounted to only 6° F. This temperature difference occurred when the flooded method of cooling was used. These results would indicate that with frequent stirring sour cream may be cooled at approximately the same rate and to about the same temperature as sweet cream in a given period of time.

#### EFFECT OF ROOM TEMPERATURE AND HUMIDITY ON THE EVAPORATION METHOD

In a previous study<sup>a</sup> it was determined that room temperature and humidity had a marked influence on the efficiency of the evaporation method. The purpose of this phase of the present study was to determine what results might be obtained when the evaporation method was used for cooling cream under various combinations of room temperatures and humidity.

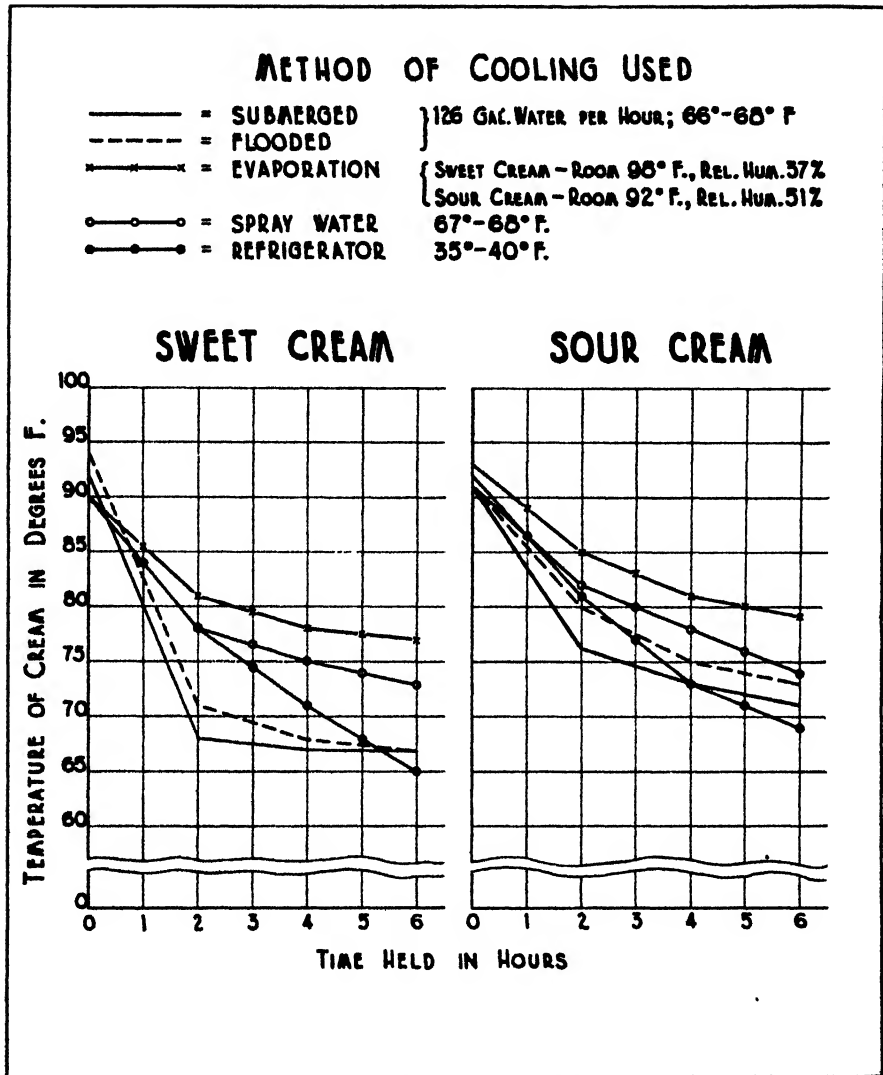


FIG. 1. Rate of temperature change in cream cooled by the following methods: flooded, submerged, evaporation, spray, and refrigerator.

In each of 10 trials (5 with sweet, and 5 with sour cream) a ten-gallon can of cream adjusted to approximately 90° F. was cooled by the evaporation method for 48 hours. The room temperatures and humidities were not artificially regulated in any of the trials. The room temperatures in the various trials ranged from 63-104° F. while the humidities varied from 25 to 68 per cent. A thermograph and hygrograph of the recording types were used to follow continuously the room temperature and humidity. The temperature of the cream was determined by means of a recording thermometer, the bulb of which was inserted and sealed into an opening in the side of the

can. A second thermometer of known accuracy was used as a check on the recording thermometer.

About six hours were required for the cream to reach the lowest attainable temperature at any given room temperature and humidity combination (Table 1). Variations in the temperature of the cream following the first six to eight hours of each trial were the result of changes in the temperature or humidity of the room or a combination of these. It was observed that fluctuations in room temperature tend to be accompanied by similar changes in the temperature of the cream but with some lag in time, which is in agreement with results previously reported.<sup>3</sup>

The temperature to which cream may be cooled by the evaporation method is definitely limited by the temperature and humidity of the room

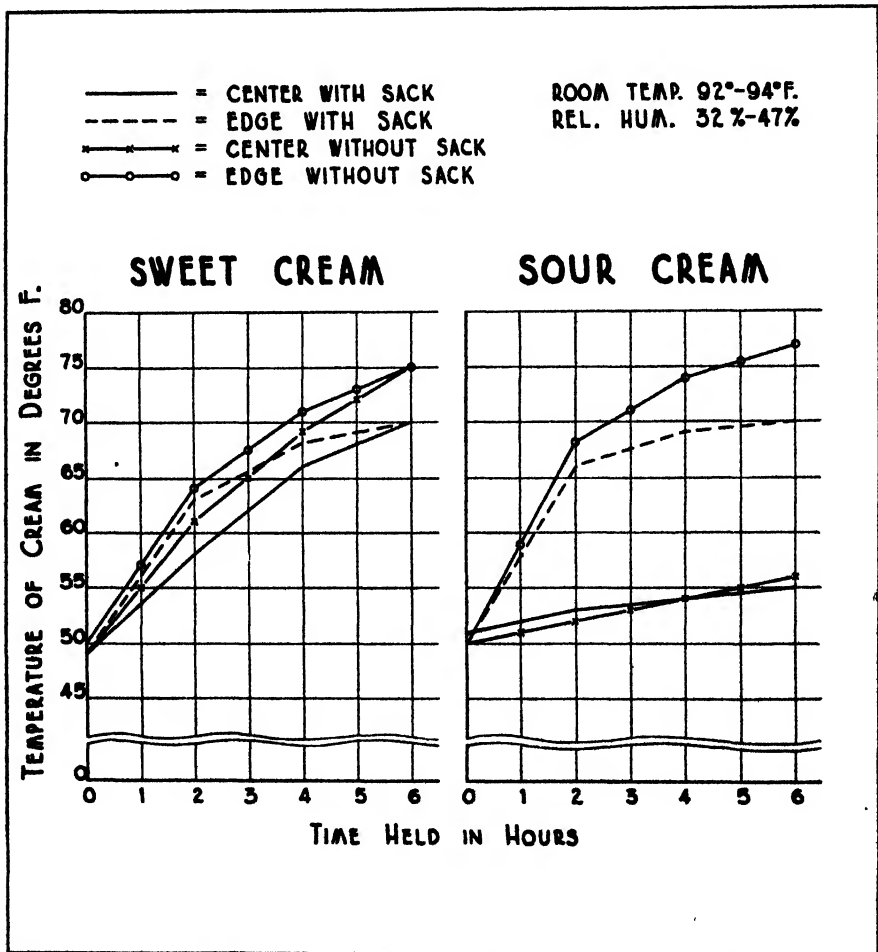


FIG. 2. Rate of temperature rise in cooled cream (35 per cent fat), with and without sacks. (Average of 3 trials with sour and 2 with sweet cream.) Room temperature 92-94° F. (Relative humidity 32-47 per cent.)

TABLE 1  
*Relation of relative humidity and room temperature to the temperature of sweet and sour cream cooled by evaporation*

TRIAL NO.	CONDITION OF CREAM	RELATIVE HUMIDITY OF ROOM (PER CENT)		ROOM TEMPERATURE IN DEGREES F.		CREAM TEMPERATURE IN DEGREES F.		DIFFERENCE BETWEEN ROOM AND CREAM TEMPERATURES	
		Ave.	Range	Ave.	Range	Ave.	Range	Ave.	Range
1	Sweet	52	39-57	103.0		86.0	82-88	17.0	15-21
2	Sour	43	39-39	103.5		83.0	82-84	20.5	19-22
3	Sweet	47	32-70	92.0	103-104	77.0	71-78	15.0	0-22
4	Sour	34	25-43	89.0	76-100	73.0	72-76	16.0	11-16
5	Sweet	46	32-60	88.0	86-94	72.0	70-74	16.0	13-20
6	Sour	63	54-68	85.0	83-87	76.0	75-78	9.0	7-10
7	Sweet	52	46-59	80.0	82-89	72.0	69-73	13.0	11-16
8	Sour	49	41-55	80.0	78-82	70.0	68-71	10.0	8-14
9	Sweet	48	45-49	72.0	66-74	62.0	57-64	10.0	9-12
10	Sour	44	41-49	71.0	68-73	56.0	55-58	15.0	13-16

(Table 1). A reduction in the temperature of the room, provided the humidity did not exceed 60 per cent, was accompanied by a reduction in the temperature to which the cream was cooled and held. When the room temperature averaged 103.5° F. for example the temperature of the cooled sour cream ranged from 82–84° F. When the room temperature averaged 89° F., however, the cream was cooled and held at 72–76° F., and when the room temperature was approximately 72° F. the temperature of the cream ranged between 57–64° F. (Table 1). Similar relationships between the room temperature and the temperature to which cream may be cooled were observed in trials with sweet cream (Table 1).

The influence of humidity on the efficiency of the evaporation method may be observed by a comparison of trials 6 and 7 (Table 1). The room temperature in both of these trials was quite uniform averaging 85° F. The humidity, however, averaged uniformly higher in trial 6 than it did in trial 7. Likewise the temperature of the cream averaged from 3 to 5° F. higher in trial 6 than it did in trial 7. Relative humidities in excess of 60 per cent appear to definitely impair the efficiency of the evaporation system of cooling.

#### EFFECTIVENESS OF THE EVAPORATION METHOD FOR HOLDING CREAM

The data obtained in the preceding trials indicate that cream cannot be cooled adequately in extremely hot weather by the evaporation method. The question arose as to how effective this system of cooling would be in preventing an undue rise in temperature in cream which has been previously cooled.

In each of five trials (3 with sour, and 2 with sweet cream) two ten-gallon cans of cream were cooled to approximately 50° F. and exposed in a room at 92–94° F. One can in each pair was protected with a sack, whereas the other can was unprotected. Since the object of this experiment was to prevent temperature changes in the cream it was not stirred until the end of the trial. Temperatures were taken at the center and edge of the cans.\*

The data show that the use of sacks on cans of cooled cream retarded the rate of temperature change in the cream (Fig. 2). The temperature of the sweet cream in the unprotected can averaged 76.5° F. at the end of seven hours exposure as compared with 70° F. for that protected by means of a wet sack. In the case of the sour cream the temperature was 75° F. in the unprotected can as compared with 70° F. in the protected can. Thus there was an average temperature difference of 6.5° F. in the cans of sweet cream and 5.0° F. in the cans of sour cream at the end of the trial in favor of the cans protected by sacks.

Had the trial been conducted over a longer period the effectiveness of the evaporation method in keeping cream cold might have been demonstrated more clearly. In the preceding trials (Table 1) it was shown that with a room temperature of over 90° F. one may reasonably expect the

evaporation method to maintain the cream about 15° F. below this temperature or at approximately 75° F. Hence the temperature of the cream in this study had not reached the point where the evaporation method would become effective at the end of the trial. It seems evident, however, that temperatures which can be maintained by the evaporation method when used for holding cream will be determined largely by the temperature and relative humidity of the room.

A comparison of the data for sweet and sour cream shows that sour cream does not warm up so rapidly nor to the same extent as sweet cream when held under similar conditions (Fig. 2). The results indicate that sour cream if delivered properly cooled may be held for longer periods under adverse temperature conditions than can sweet cream without an undue change in temperature.

#### DISCUSSION

Of the various methods of cooling compared in this study the use of a refrigerator box maintained at 35–40° F. would approach the ideal condition for the storage of cream. The practical difficulty of providing such facilities in the average cream station, however, would definitely limit the use of this method to a very few of the larger stations and creameries receiving their cream direct.

If an ample supply of cold water is available the use of a cooling tank through which cold water flowed continuously would be a more practical, efficient, and convenient system of cooling than either the spray or flooded systems. When the spray method is used, cooling is accomplished primarily by the conduction of heat away from the can by cold water and very little if any is due to the evaporation of the water. Furthermore, it has been reported<sup>8</sup> that considerable heat is absorbed from the surrounding atmosphere by the spray water when this method is used, thereby raising the temperature of the water which is used for cooling. There is no appreciable absorption of heat from the surrounding atmosphere by the cooling water when allowed to flow continuously through a cooling tank in which the cans of cream are placed. Although the flooded system as used in this study proved to be practically as efficient as the use of a cooling tank, the latter method would be more practical and convenient to use. The feasibility and practicability of using any method of water cooling would be dependent upon the availability of running water at reasonably low temperature.

Although the evaporation method cannot be relied upon to cool cream adequately in exceeding hot weather, the use of this method would certainly be better than no cooling at all. The method is best adapted for room temperatures not in excess of 85° F. and humidities not in excess of 50 per cent. Under such conditions it should be possible to cool and maintain cream at approximately 70° F. or below. Cream maintained at 70° F. will remain in the first grade much longer than that which is held at 85° F. or above.

## SUMMARY AND CONCLUSIONS

A study has been made to compare the relative efficiency of five different methods of cooling cream including: (1) placing cans of cream in a refrigerator, (2) partially submerging the cans in a cooling tank containing continuously flowing water (submerged), (3) allowing water to flow over the outside of the can (flooded), (4) use of spray, and (5) use of the evaporation method. In addition the effectiveness of the evaporation method of cooling sweet and sour cream through a wide range of atmospheric temperatures and humidities was determined. The feasibility of using the evaporation method of cooling in preventing a rise in the temperature of cream which had been previously cooled was also studied.

The results obtained in this study may be summarized as follows:

1. The rate of cooling during the first two hours of the trial was fastest by the submerged method, followed in order by the flooded, refrigerator, spray, and evaporation methods of cooling. At the end of the six-hour trial the cream placed in the refrigerator was cooled to a slightly lower temperature than that cooled by either the flooded or submerged methods of cooling and to from 5–12° lower than the cream cooled by either the spray or evaporation method. The spray system was slightly more efficient than the evaporation method under the conditions of this trial (room temperature 92–98° F.).

2. Sour cream, irrespective of the cooling method used, did not cool so rapidly nor to quite so low a temperature as did sweet cream.

3. It required about six hours for either sweet or sour cream to reach the lowest attainable temperature under a given set of conditions, when cooled by the evaporation method.

4. The temperature to which cream may be cooled by the evaporation method was definitely limited by the temperature and humidity of the room. When the room temperature averaged 103.5° F. sour cream was cooled to 82–84° F. When the room temperature averaged 89° F., however, the cream was cooled to 72–76° F., and when the room temperature was approximately 72° F. the temperature of the cream was reduced to 57–64° F.

5. If the relative humidity is held rather constant, fluctuations in the room temperature tend to be accompanied by corresponding changes in temperature of the cream, but with some lag in time when the evaporation process is used for cooling.

6. Efficiency of the evaporation system of cooling was impaired when the relative humidity exceeded 60 per cent.

7. The evaporation method retarded the rate of temperature rise in cooled cream and prevented the temperature of the cream from going above that attainable by this system of cooling. The temperature rise was less rapid in sour than in sweet cream indicating that viscous sour cream was a poorer conductor of heat than sweet cream.

# A RAPID PHOSPHOMONOESTERASE TEST FOR CONTROL OF DAIRY PASTEURIZATION

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Although health authorities seek to carefully define the requirements of pasteurization by means of ordinances, they are repeatedly confronted by failures to comply with regulations. The human element involved in the pasteurizing process and many of the mechanical factors contributing to improper pasteurization cannot be satisfactorily controlled by routine physical inspection of plants. Temperature recording devices may become inaccurate or inoperative between the visits of plant inspectors. Some defects are apparent only to an inspector who has studied the many and complex types of pasteurizing systems and individual equipment utilized. Other irregularities cannot be detected visually.

The holding process of pasteurization (which is the most widely used in this country) as required by the New York City Sanitary Code consists of heating the milk to at least 143° F. and holding it at that temperature for at least 30 minutes, after which it is rapidly cooled. In many cases, however, there is not always assurance that all the milk is held for the desired time or that the proper temperature has been employed. Properly pasteurized milk may be contaminated with under-heated foam (1). Without agitation in the holding tank, there is always the risk of non-uniform temperature distribution. In this connection we note the New York State definition (Sanitary Code Regulation 22): "No milk or cream shall be labeled or designated as pasteurized unless every particle of such milk or cream has been subjected to a temperature of 143° F. or more, continuously for not less than 30 minutes. . . ." The Federal definition also emphasizes *every particle*.

Chemical tests thus far proposed for the detection of improperly or inefficiently pasteurized milk may be grouped for simplicity based on:

1. Microscopic examination: Cellular test of Frost (2).
2. Physical properties: Creaming test (3), Turbidity (4), Cheesing (5), Lactalbumin (6), Suspensions (7).
3. Enzyme activity: Peroxidase (8), Catalase (9), (10), Aldehydereductase (11), Amylase (8), (12), (13), and Phosphatase (14).

With the advent of low temperature pasteurization many of these tests have been inapplicable or are not satisfactory (6, 8, 12, 15, 16, 17, 18, 19, 21, 22, 23). There seems to be a personal factor or equation in almost all the methods thus far reported which exposes them to the criticism of failure at duplication by other workers. Early in 1935, this writer developed a

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modified amylase test which in some thousands of applications always distinguished raw milk as such and indicated the presence of 5 per cent of added raw milk, or an over-all heating period of fifteen minutes or less, or when a temperature of 140° F. or less had been employed. Results obtained from the use of this modified method were encouraging, and indicated that a more sensitive method was needed.

The recently proposed phosphatase test (14) offers greater sensitivity but is somewhat lengthy and involved for routine analysis. Although Kay and Graham suggested that for American temperatures of pasteurization ( $142.5 \pm 2.5^\circ$  F.) 2½ hours of incubation were sufficient, Gilcreas and Davis (20) found that 24 hours were required for fine distinctions on the quantitative test. Results in this laboratory are in agreement on that point. Critical preparation of the Folin and Ciocalteu reagent was essential and somewhat expensive. Furthermore, this reagent is lacking in specificity, reacting with many substituted phenols and even with tin and ferrous iron (24, 25).

In January, 1937, the application of various protein precipitants in conjunction with other phenol reagents was investigated and in this connection it was found that basic lead acetate when used with 2,6 dibromoquinone-chloroimide was entirely satisfactory. The sensitivity of this reagent (hereinafter termed "BQC") to phenol is one part in twenty million. It reacts with only those phenols in which the position part to the hydroxyl group is unsubstituted to form indophenols giving rise to blue colorations upon which a quantitative estimation of phenols may be based (26). A technique devised to fit the requirements of this reagent is herewith presented which is short, inexpensive, uses only such equipment as can be found in the average health department or dairy laboratory, and is reasonably devoid of personal equation. Although only a limited number of determinations (about 10,000) were made, excellent checks were obtained and the method seems worthy of further detailed study.

Application is made of the destruction of phosphomonoesterase,<sup>1</sup> a constant constituent of raw milk, by heat treatment, 96 per cent being destroyed at 143° F. in 30 minutes (14) and the ability of this enzyme to hydrolyze disodium phenylphosphate (27) with the liberation of phenol which is estimated colorimetrically with 2,6 dibromoquinonechloroimide. The test is conducted in an alkaline medium about pH 9.6 which is optimum for the development of coloration. At a lower pH, development is unduly prolonged while at a higher pH color may fail to develop (26). The greater the amount of phenol present, the more rapid the development of color; excess of the

<sup>1</sup> Folley and Kay (28, 29, 32) classify the phosphatases, terming as alkaline phosphomonoesterase the phosphatase in milk which is active at an alkaline pH. It seemed wise at this point to particularize on this name in order to differentiate the qualities of this enzyme from other phosphatases.

imide also speeds formation of the indophenol but too great an excess with small amounts of phenol add yellow to the blue.

#### THE MODIFIED PHOSPHATASE METHOD

##### *Reagents*

**2,6 Dibromoquinonechloroimide** (Referred *infra* as BQC) (Eastman Kodak Chemical No. 2304)

Dissolve 0.04 gram in 10 ml. of 95 per cent ethyl alcohol. When tightly stoppered this solution is stable for several days. It was found convenient to keep this solution in an Owens  $\frac{1}{4}$ -ounce green glass pharmaceutical dropper bottle (Design No. 90943). This size dropper bottle delivers 50 drops to the cubic centimeter of the alcoholic solution of the imide. Two drops or  $\frac{1}{25}$  ml. of the imide contain 0.16 milligram. This has been indicated as an adequate excess.

**Basic Lead Acetate** (prepared by the Horne Method) A.C.S. specifications

Boil 280 grams of the dry basic lead acetate with 500 ml. of water for 5–10 minutes. Cool, allow to settle, filter and dilute to 500 ml.

**Borate Buffer Solution**

Dissolve 28.427 grams of  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10 \text{H}_2\text{O}$  (analytical grade) in 900 cc. of warm distilled water. Stir vigorously while powder is being added to prevent lumping. Add 3.27 grams of NaOH in the form of a strong solution (2 to 5 Normal), cool and make up to a liter.

**Buffered Substrate**

The Disodium phenyl phosphate obtained from British Drug Houses Ltd., was found to contain considerable free phenol.<sup>2</sup> Accordingly the salt was washed with ethyl ether until the washings gave a negative test for phenol. In making this test, add 10 ml. of distilled water to 100 ml. of the ether washings, evaporate off the ether, add  $\frac{1}{2}$  ml. of the borate buffer, then add 4 drops of BQC. The development of a blue color indicates the presence of phenol.

The washed salt should be air dried briefly to remove the ether, then dried in a desiccator. Store in a refrigerator.

Dissolve 1.09 grams of the washed and dried salt in 900 ml. of distilled water previously saturated with  $\text{CHCl}_3$ . Add 50 ml. of borate buffer solution. Make up to 1 liter. Add a few drops of  $\text{CHCl}_3$ . Store in refrigerator. The pH of this buffer substrate is about 9.6 and should be checked using Thymolphthalein as an indicator.

##### *Phenol Color Standards*

Prepare a solution of phenol in distilled water standardized at 0.1 mg. per ml. (80). Dilute the required number of milliliters to 100 ml. to give solutions containing  $\frac{1}{2}$ , 1,  $1\frac{1}{2}$ , 2, 3, 4, 5, 10, 20, 50 and 100 parts per million. To make color standards, take 5 ml. portions of these solutions, add  $\frac{1}{2}$  ml.

<sup>2</sup> This salt suitably phenol free may be obtained from Eimer & Amend, New York, or R. P. Cargille, New York.

borate buffer and two drops of BQC solution. Stopper tightly. These color standards are stable for several weeks if stored under refrigeration and kept out of direct sunlight when used.

It is proposed for convenience to identify as the unit of blue, the amount of color produced by 0.001 mg. of phenol per 5 ml. of solution. Thus the standard containing  $\frac{1}{2}$  p.p.m. phenol would contain 0.0025 mg. (2.5 gamma) of phenol per 5 ml. or 2.5 units, the standard containing 1 p.p.m. would contain 0.005 (5 gamma) per 5 ml. or 5 units, etc.

### *Method*

A blank should be run on the reagents daily. If the blank produces any blue color, the solution of buffer substrate should be discarded.

By means of a pipette (a different pipette for each sample) transfer 1 ml. of the milk to be tested to a pyrex test-tube. (The pipette should be plugged with cotton to prevent contamination with saliva. It was found convenient for rapid work to use a pipette not graduated to the tip so that no drainage was required.) Add 10 ml. of the buffer substrate. Shake well. Incubate for one hour at 37.5° C. After incubation, place tubes in boiling water for 5 minutes. Cool in ice water. Add 0.1 ml. of Basic Lead Acetate solution. Shake well. Allow to stand for a minute or two. The proteins will coagulate and separate sharply (in some instances it may be necessary to add an additional 0.05 ml. of the lead acetate to completely precipitate the protein). Filter. To 5 ml. of the clear filtrate add 0.25 ml. of the borate buffer. Add 2 drops or 0.04 ml. of BQC. Shake gently.

The blue color of the indophenol reaction develops within five minutes; the greater the amount of phenol present the more rapid the development. Allowing fifteen minutes for development, the color adduced is then compared with the phenol color standards and reported as phosphomonoesterase units—a unit being the amount of enzyme which under conditions of the test would produce the color equivalent of 1 gamma of phenol.

### *Precautions To Be Observed in Making the Laboratory Test*

(A) Samples of milk and cream, especially if stored under refrigeration, should be warmed to room temperature before pipetting.

(B) If an incubator rather than a water bath is utilized, it is suggested that the test-tubes, before being placed in the incubator, be placed for a minute or two in water at a temperature of about 40° C.

(C) Occasionally following the use of the basic lead acetate, the filtrate is not water clear, or the filtrate becomes clouded as a result of the addition of  $\frac{1}{4}$  cc. of the borate buffer. Centrifuging at a speed of about 800 r.p.m. will serve to clarify the serum. The precipitate does not seemingly interfere with the indophenol development.

(D) Whatman No. 40 filter paper was utilized in the investigation but for routine work any good quality of filter paper will serve.

(E) Suitable precautions must be taken to prevent the action of human salivary phosphatase.

## EXPERIMENTAL DATA

*Effect of Variation in Pasteurization Time and Temperature*

Samples of mixed herd raw milks were heated in the laboratory under various times of holding at 143° F. and examined. A brief study was also made of the effect of variation in holding time in commercial plants. Samples were taken by inspectors under as nearly identical conditions as possible from four different pasteurizing plants representing two small size batch pasteurizers and two multiple unit automatic holders.

TABLE 1

*Effect of time held at 143° F. expressed in proposed phosphomonoesterase units*

	LABORATORY HEATED SAMPLES (PREHEATING 1 MINUTE)		
	Minimum	Maximum	Weighted average
Raw milk	500 Units	> 500	> 500
5 minutes heating	100	250	100
10 " "	20	50	35
15 " "	15	20	17.5
20 " "	10	10	10
25 " "	5	5	5
30 " "	0	< 2.5	< 2.5

	SAMPLES FROM COMMERCIAL PASTEURIZING PLANTS			
	Plant A	B	C	D
Raw milk	> 500 Units	> 500	> 500	> 500
5 minutes heating	40	50	100	50-100
10 " "	20	20-25	40	50
20 " "	10	10	15	25
25 " "	2.5-5	2.5-5	5-10	5-10
30 " "	2.5	< 2.5	< 2.5	< 2.5
Routine bottled milk	2.5	< 2.5	< 2.5	< 2.5

*Plant A.* Milk preheated in I-T heater approximately 60 seconds. Held in multiple automatic precision holder.

*Plant B.* Milk preheated in I-T heater approximately 60 seconds. Six and a half minutes required to fill holding tank. Six Cherry Burrell Spray holders. Three minutes required to empty holding tank. Milk cooled to 50° F. after holding period, in 30 seconds.

*Plant C.* Small batch pasteurizer. Milk required preheating period of 15 minutes before temperature of 143° F. was reached. Cooled after pasteurization by surface cooler.

*Plant D.* Small batch pasteurizer with continuous agitator. Preheating time in vat was 15 minutes. Cooled in vat five minutes.

It should be noted in connection with the above commercial plant samples (Table 1) that time indicated may not have been exact. Some samples received an additive holding time of 143° F. necessitated by the time required to fill the holding tanks, following which the holding period starts. Raw milks from each of 34 cows gave a value of 500 or more phosphomonoesterase units. In no instance did properly pasteurized milk (laboratory or commercial plant) yield a color indicative of more than 2.5 phosphomonoesterase units. Experience has shown that this value is somewhat generous in that

properly pasteurized samples uniformly show less than this amount of enzyme activity. There is no question of the ability of the test to discriminate between raw and pasteurized milk. Variations of five minutes or more in the holding time can be readily distinguished. Milk heated for 25 minutes can be distinguished from pasteurized milk. The reaction of milk heated for 5 minutes can be distinguished from raw milk. The results in Table 1 apparently show that the enzyme in question is more effectively destroyed in an automatic precision holder than in the older type batch pasteurizer and that quick preheating of the milk was more effective in destroying the enzyme than slow preheating. These indications however are based on a very limited number of determinations.

Samples of mixed herd milk were heated for 30 minutes in the laboratory at various temperatures (Table 2). The results are summarized.

TABLE 2  
*Effect of variation in temperature of heating for 30 minutes, expressed in proposed phosphomonoesterase units*

TEMPERATURE	AVERAGE VALUE
° F.	
138	250-50 units
140.5	100 "
142	10 "
143	2.5 "

The effect of the addition of varying proportions of raw milk to properly pasteurized milk is expressed in Table 3. It will be noted that the addition of 0.5 per cent of raw milk to properly pasteurized milk could be readily determined.

Experience in the past six months of both laboratory prepared and commercially heated milk has shown that whenever the value obtained as a result of this method exceeded 2.5 units, said milk was underpasteurized or improperly pasteurized and that the greater the number of units obtained, the more improper the conditions under which such milk was processed. It is not possible however, as a result of routine examination, to correctly designate whether this condition be due to either a variation in holding time, or a drop in temperature, or the addition (through leakage or improper design of equipment) of raw or improperly pasteurized milk, or contamination with underheated foam, or any combination of the foregoing. Experience further demonstrates that it is not necessary to run a control for each sample. If this be desirable in order to eliminate the possibility of phenol or phenolic products which would react with BQC being present in the milk sample originally, it can readily be performed by the substitution of a water solution (buffered to a pH of 9.6) for the buffer substrate solution and continuing either with the method as outlined previously or the quick "field" test as outlined subsequently in this article. It might be of further interest to state that even where commercially so called "pasteurized" milks developed 500

units as a result of incubation with the substrate, the control gave a negative result for phenol.

### EFFECT OF STORAGE CONDITIONS

It has been indicated (14, 20) that when milk has soured or putrefied during storage, an increase in the color value might be obtained in the Kay and Graham test due to the action of certain bacteria or to protein decomposition which might liberate phenol or phenol-like products. Such a condition might classify a properly pasteurized milk as an underpasteurized product. Some of these decomposition products such as Tyrosine and Tryptophane will give blue color with the Folin and Ciocalteu reagent (25). A view at the structural formulae of these and related compounds indicates that the position para to the OH group has already been substituted or that the compound does not contain an OH group, in either case theory suggests that there would be no reaction with BQC to form the indophenol.

Forty-two samples obtained from several commercial plants (under various holding times at 143° F.) and 22 laboratory-prepared samples (representing variations in holding time, in temperature and mixtures of raw and pasteurized milk) were allowed to age under storage conditions of refrigeration, and tested from time to time. Tables 3, 4, 5 and 6 indicate the results of this study.

Improperly pasteurized milks were indicated after a considerable lapse of time. The apparent decrease in phosphomonoesterase units may have

TABLE 3

#### Results under storage conditions of the addition of raw milk to pasteurized milk:

PERCENTAGE OF RAW MILK ADDED	UNITS OF PHOSPHOMONOESTERASE		
	1 day	7 days	14 days
0.0	< 2.5	< 2.5	< 2.5
0.2	< 5	< 5	< 5
0.5	5	5	5
1.0	10	> 10	< 10
2.0	15-20	> 10	> 10
5.0	20-50		
10.0	100		

TABLE 4

#### *Effect of storage conditions of commercial pasteurized milk on the test*

[illegible]

TABLE 5

*Effect of storage conditions on samples heated less than 145° F. for 30 minutes, expressed in proposed units*

TEMPERATURE	AGE IN DAYS					TITRED WITH Na <sub>2</sub> CO <sub>3</sub> BEFORE SAMPLING AFTER 27 DAYS
	1	3	6	10	27	
°F.						
138	500	500	100	100	10	50-100
140.5	100	50-100	50	15	5-10	20
142	10	5-10	10	5	2.5	2.5-5
143	2.5	2.5	2.5	2.5	2.5	2.5

TABLE 6

*Results of souring under storage conditions, expressed in proposed units*

	SAMPLE NUMBER	AGE IN DAYS				
		1 day	7 days	10 days	20 days	50 days
Raw milk	1	500	500		500	500
	2	500			500	500
	3	500		500		500
	4	500	500	500	100 +	
Preheated	5 A	500				500
	6 B	500			500	100
	7 C	500				500
	8 D	500				
Held 10 minutes at 143° F.	9 A	15	15	15		5
	10 A	40	40	40		20
	11 A	25	20	10	5	5
	12 C	50	50	50		
	13 D	20	20	20		10
	14 A	20	25	20	15	
Held 20 minutes at 143° F.	15 A	25	25	20		
	16 B	5				2.5
	17 B	10	10	<10		<5
	18 B	5	5	2.5	2.5	
	19 C	25	20			2.5
	20 D	10	10	5		2.5
	21 A	10	10	10	5	
	22 A	10	10	10	5	

A = Laboratory preheating 1 minute.

B = Commercial plant " 1½ "

C = " " " 15 minutes.

D = " " " 15 " accompanied by agitation in tank.

been due to the increase in acidity coupled with the inadequacy of the buffer under such extreme conditions to maintain the optimum pH for activity of the enzyme. Moreover due to the condition of the milks, it was difficult to obtain a representative sample. A portion of some of the milks were titred with Na<sub>2</sub>CO<sub>3</sub> to an approximate pH of 6.6 before sampling and proceeding with the analysis. The results obtained in this fashion are of considerable interest.

#### APPLICATION OF THE TEST TO MILK PRODUCTS

Samples of raw cream and samples of commercially pasteurized cream from reliable plants were subjected to the test. No change in technique was

found necessary. Raw creams gave a value in excess of 500 units while pasteurized cream gave a value of 2.5 units (in two instances 5 units).

Chocolate milk could also be analyzed with no change in technique. Experiments now being conducted indicate that butter and other milk products may be examined with little change in the method but because of the very limited number of samples analyzed no data is presented.

#### SHORT-TIME, HIGH TEMPERATURE PASTEURIZATION

Samples of milk obtained from a York high temperature pasteurizer reacted to the test in the same fashion as normally pasteurized milk. This equipment was rated to heat the milk at 160° F. for 15 seconds. The length of holding when checked with water was found to be 17 to 18 seconds.

#### APPLICATION OF THE PROPOSED TEST TO COMMERCIALY PASTEURIZED MILKS AND CREAMS

While it would not be feasible to tabulate the findings on the many samples analyzed, Table 7 indicates some representative results obtained on market samples of milk and cream labeled pasteurized. It will be noted that two classes of samples are considered: routine samples and official samples. The routine samples were submitted primarily for milk fat and total solids determinations, but were also subjected to the pasteurization test. When the result obtained on such sample indicated the possibility of improper pasteurization, an official sample was obtained that same day and submitted solely for analysis by this test. In many instances, inspectors equipped with the "field" test would visit the plant concerned during operation that night, and check operating conditions. Existing improprieties in technique or shortcomings of apparatus were uncovered and demonstrated to the plant operators.

TABLE 7

*Selected results obtained on market milk and cream samples designated as pasteurized*

NATURE OF SAMPLE	PROPOSED UNITS	REMARKS
Routine cream ..	100	Improperly pasteurized
Official milk	20	Pumps not regulated, by-pass permitted, holding time improper
Official cream ...	500	Plant records indicated pasteurization of 20 per cent. more than capacity
Official milk . . . .	15	Holding period of 20 minutes
Official milk . . . .	15	Thermometer 2° off
Official cream . . . .	10 }	Cream standardized after pasteurization
Official cream . . . .	25 }	with raw or improperly pasteurized milk
Official milk . . . . .	7.5 }	Excessive foam in vat
Routine cream . . . . .	7.5	Agitators not operated during holding period
Routine milk . . . . .	15	Temperature less than 142° F.

The point has been made (33) that slow cooling of milk held at the pasteurizing temperature range for 20 to 40 minutes resulted in reactivation

of some of the phosphomonoesterase, and that with rapid cooling the phosphatase destruction is much greater. This has not as yet been confirmed by the author's experiments but should present no problem since proper pasteurization requires the quick cooling of the milk.

Folley and Kay have shown (31) that the phosphomonoesterase content of cow's milk varies during lactation, rising to a maximum 180 days post partum. There may also be a variation in the enzyme content of the milk of individual cows but this is hardly material when dealing with market milk which is usually mixed or herd milk. They further indicated (29) that the amount of the enzyme present in raw milk bears a relation to the solids not fat.

At present a study is being made to see whether a longer incubation period will increase the sensitivity of this test or aid its diagnostic value. However, since the speed with which hydrolysis occurs decreases with an increase in reaction time and since the activity of a phosphatase solution is not proportional to its concentration, it would seem better to use a short incubation period in which enzymic activity is nearer to true initial velocity of hydrolysis. The catalytic effect of magnesium ions (32) on the sensitivity is also being investigated.

#### A SHORT FIELD TEST

Because of its simplicity, the procedure previously suggested as a laboratory method, could be used in the field. However it developed that the blue color of the indophenol reaction would appear even in the presence of the milk proteins and fat. The following method was evolved as a field test utilizing the compact fabricoid kit (obtained from R. P. Cargille) shown in Figure 1.

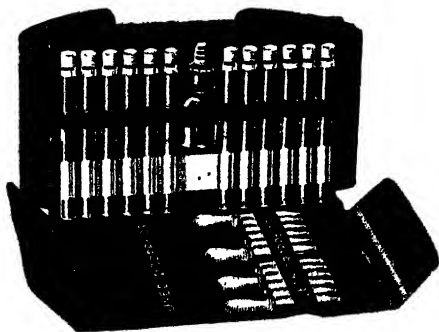


FIG. 1. The compact field kit. Test-tubes are flat bottom, each calibrated at 5 ml. and 5.5 ml. Buffered substrate is added up to lower mark on tube, sample is then added by means of dropper to higher calibration.

To 5 ml. of buffered substrate add 0.5 ml. of sample to be tested. Stopper, shake well. Incubate for 5 to 10 minutes in water at about 100° F. or by placing tube in vest pocket. Following incubation add 6 drops of BQC.

Stopper and shake. Note color after 5 minutes. The appearance of any blue is indicative of improper pasteurization.

Tablets suitable for the preparation of the two necessary solutions are available.

## APPLICATION OF THE FIELD TEST

TABLE 8

SAMPLE	INTENSITY OF BLUE COLOR
Raw milk	+ + + +
Milk preheated at 120° F.	+ + + +
“ “ “ 143° F.	+ + +
Milk held 10 minutes at 143° F.	+ +
“ “ 15 “ “ “ “	+
“ “ 20 “ “ “ “	±
“ “ 30 “ “ “ “	-
5% raw milk + pasteurized milk	+ +
2% “ “ + “ “	+
1% “ “ + “ “	±
Raw cream	+ + + + +
Pasteurized cream	-

The indophenol blue develops within 5 minutes and becomes progressively more intense. Properly pasteurized cream yields a grey to white color, while properly pasteurized milk gives a grey to brown appearance. It has been demonstrated that whenever the laboratory test would indicate 10 units or more, the field test would also indicate blue. In some cases the field test reacted as positive on cream when the laboratory test showed only 7.5 units of color. It has even demonstrated the contamination of a pasteurized product with underheated foam. Where the results obtained on this field test are questionable, a sample should be subjected to the more sensitive laboratory test.

The following instance will serve to demonstrate the practical application of the two tests: Cream from plant X in the city was reported to yield a high unit value on the laboratory test. This plant merely bottled bulk “pasteurized” cream which it received from about 20 different country plants. Inspectors using the field test isolated the source responsible. This lot gave a very high phosphomonoesterase reaction, and led to the testing and embargo of a subsequent delivery of a large shipment of cream. It was then shown by the laboratory test that this large quantity of bulk cream was not uniform in its reaction, falling roughly into three classes: 500 units, 100 units and 20 units. An inspector of the Country Milk Division using the field test checked the plant concerned, found certain temperature irregularities in one tank (chart indicated 143° F. but due to inadvertent slipping of the bulb of the recording thermometer so that it rested on the metal bottom of the pasteurizing vat, the actual temperature was only 139° F.); this fact plus lack of agitation and the further observation that this “pasteurized” cream was standardized when necessary by the addition of milk which in turn

was improperly pasteurized, may have accounted for the variation in the amounts of phosphomonoesterase units found. The operative irregularities were corrected and the cream now uniformly indicates less than 2.5 units.

#### CORRELATION BETWEEN BACTERIA COUNTS AND PHOSPHOMONOESTERASE UNITS

Several thousand commercially pasteurized milks and creams upon which bacteria counts were made were analyzed for phosphomonoesterase activity.

The data are not presented as it is evident that factors other than the thoroughness of pasteurization greatly affect bacterial counts. There were a number of samples giving high bacteria counts with a phosphomonoesterase value of 2.5 units. This may have been due to high initial bacterial content of the raw milk, to resistant bacteria, to improper storage, or to the age or the subsequent contamination of the products. However, a low count milk is not always a safe milk.

The phosphomonoesterase value of a sample gives no indication of bacterial contamination of a product subsequent to pasteurization.

#### DISCUSSION OF PRACTICAL VALUE OF THE TEST

During the first month of application of this test, reporting as abnormal (*i.e.*, reacting positive) only those milks or creams whose phosphomonoesterase value exceeded 5 units, and applying the rough field test to the sources or plants thus indicated, 23,977 pounds of milk and cream were condemned as improperly pasteurized and excluded from the New York City market.

It is evident as a result of the work done thus far that faulty pasteurization falls into two classes: unintentional and deliberate. The unintentional class may be due to:

- (a) Poor or inadequate equipment.
- (b) Careless use of good equipment.
- (c) Failure to check operating conditions of the apparatus.
- (d) Failure to check plant thermometers against a Bureau of Standards thermometer.
- (e) Failure to eliminate excessive foaming.
- (f) Fluctuation in temperature due to manual control.

The deliberate class may be subdivided into:

- (a) Deliberate dropping of temperature to 140° F. or lower in order to produce an apparently increased "cream line."
- (b) Non-operation of agitators for the above reason.
- (c) Cutting down on the holding interval so as to fill the plant flow sheet requirements during a temporary breakdown of part of the equipment.
- (d) Laxity in operating conditions on the last run of milk.
- (e) Deliberate omission of the holding period.

(f) Addition of raw milk to pasteurized milk in order to fill quota, or standardization of cream with raw milk.

(g) Uncontrolled foaming.

#### SUMMARY

A review of the literature is given and a rapid method is described for the control of pasteurization of milk, cream and related milk products. Enzymic hydrolysis of a substrate is measured quantitatively by the addition of 2.6 dibromoquinonechloroimide resulting in indophenol blues of varying intensities if faulty pasteurization is present. The test can be completed in less than an hour and a half and will demonstrate such minor faults in technique of pasteurization as:

(1) A temperature of 1° F. below the 143° F. level required by the New York City Sanitary Code.

(2) Heating for 25 minutes instead of the required 30 minutes at 143° F.

(3) The addition of 0.5 per cent of raw milk to properly pasteurized milk or cream.

The method requires no special apparatus. It is inexpensive and may be used in plant control as an index of the efficiency of pasteurization. A method of reporting results in proposed phosphomonoesterase units is suggested.

A simple field test which can be completed in approximately ten minutes and which can be used by an inspector is reported which presents definite diagnostic value.

#### ACKNOWLEDGMENT

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# THE SURVIVAL OF PATHOGENIC MICROORGANISMS IN ICE CREAM

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The results of a study of the longevity of *Brucella*, *Mycobacterium*, and *Salmonella* species in ice cream has been reported previously by Wallace and Crouch.<sup>1</sup> In that study cultures of *Salmonella aertrycke*, *Salmonella enteritidis*, *Brucella abortus* Bang, *Brucella abortus porcine*, *Brucella melitensis*, *Mycobacterium tuberculosis hominis* (strains A, I & S), *Mycobacterium tuberculosis bovis* and *Mycobacterium avium* were inoculated and thoroughly mixed into vials of commercial ice cream and frozen at  $-23.2^{\circ}\text{C}$ . ( $-10^{\circ}\text{F}$ ). The *Brucella* and *Salmonella* species were put into the ice cream in the spring of 1930 and the *Mycobacterium* species in the fall of 1931. The vials containing *Brucella* and *Salmonella* species were held for 36 months and those containing *Mycobacterium* species for 30 months with periodic examinations to determine the viability of the inoculated organisms. At the end of those periods of storage all the species were found to be viable. A short review of the literature was also reported. Since that time Thompson<sup>2</sup> examined ice cream made from naturally infected cows' milk for the presence of *Brucella*. He found that the *Brucella* were carried into the ice cream during the manufacturing process and that they existed there for at least one month.

At the time of publication of the results by Wallace and Crouch there were a few vials of each series remaining, so it seemed desirable to continue holding them in storage to see how much longer the inoculated organisms would survive. Consequently, samples were examined in the spring of each year from 1934 through 1937. The same procedures mentioned in the first publication were again used. Where it was necessary to use animals three guinea-pigs were inoculated with each sample of ice cream.

The results, given in Table 1, do not seem to need very much discussion. Because the ice cream probably received a heavy inoculation of microorganisms and because commercial ice cream is made from pasteurized mix and is usually held for a few days only the practical importance of these results is not to be emphasized. However, it is interesting to observe that the microorganisms studied will survive for such a long time in ice cream at  $-23.2^{\circ}\text{C}$ . ( $-10^{\circ}\text{F}$ ).

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<sup>1</sup> Wallace, G. I., and Crouch, Rhoda. Microbiology of frozen foods. VI. The survival of pathogenic microorganisms in ice cream. *JOUR. DAIRY SCI.* 16: 315. 1933.

<sup>2</sup> Thompson, E. The isolation of *Brucella abortus* from ice cream. *Canadian Med. Assoc. Jour.* 29: 9. 1933.

TABLE 1  
Showing the viability of microorganisms stored in ice cream

Year	1930-33	1934	1935	1936	1937
Years of storage	3	4	5	6	7
ORGANISMS					
<i>Salmonella aertrycke</i> . . . . .	++	++	++	++	-
<i>Salmonella enteritidis</i> . . . . .	++	++	++	++	+
<i>Brucella abortus</i> Bang . . . . .	++	++	++	++	++
<i>Brucella abortus porcine</i> . . . . .	++	+	-	-	-
<i>Brucella melitensis</i> . . . . .	++	++	+	-	-
<i>Mycobacterium tuberculosis hominis</i> A . . . . .	++	++	++	++	++
<i>Mycobacterium tuberculosis hominis</i> I . . . . .	++	++	++	++	++
<i>Mycobacterium tuberculosis hominis</i> S . . . . .	++	++	++	++	++
<i>Mycobacterium tuberculosis bovis</i> . . . . .	++	++	++	++	++
<i>Mycobacterium avium</i> . . . . .	++	++	++	-	-

++ in *Salmonella* = numerous colonies on culture.

++ in *Brucella* and *Mycobacterium* = all animals positive.

+ in *Salmonella* = few colonies on cultures.

+ in *Brucella* and *Mycobacterium* = at least one animal positive.

- = no viable organisms demonstrated.

#### SUMMARY

*Salmonella enteritidis* and *Brucella abortus* Bang have survived storage in ice cream at a temperature of  $-23.2^{\circ}\text{C}$ . ( $-10^{\circ}\text{F}$ .) for 7 years. Of the remaining organisms *Mycobacterium tuberculosis hominis* (strains A, I & S) and *Mycobacterium tuberculosis bovis* have survived for  $6\frac{1}{2}$  years, *Salmonella aertrycke* for 6 years, *Brucella melitensis* for 5 years, *Mycobacterium avium* for  $4\frac{1}{2}$  years and *Brucella abortus porcine* for 4 years. It should be made clear that *Salmonella enteritidis*, *Brucella abortus* Bang, *Mycobacterium tuberculosis hominis* (strains A, I & S) and *Mycobacterium tuberculosis bovis* were still viable and that later tests will be necessary to determine their longevity.

# THE FUNCTIONAL ACTIVITY OF THE RIGHT AND LEFT BOVINE OVARY<sup>1</sup>

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The statement is made quite frequently that the right ovary of the bovine ovulates more frequently than the left ovary. However, very meager data are presented in support of the statement.

For a review of the earlier reports on the alternating action of the ovaries the reader is referred to Hammond (1). During the years 1907 to 1915 Stalfors (2) made rectal examinations of pregnant cows for the purpose of determining in which horn of the uterus the fetus was carried. Out of a total of 923 cows examined, 577 (62.5 per cent) carried the fetus in the right horn of the uterus and 346 (37.5 per cent) in the left horn. One hundred and five of these cows were kept under observation for two successive gestation periods and in 62 cows the fetus was twice carried in the same horn. Hammond (1) observed that in 65 per cent of the animals he examined the follicle ripened in the opposite ovary to that in which the previous ovulation occurred. Investigators at the Idaho Agricultural Experiment Station (3) reported that out of 146 pregnancies, determined by rectal examination and later proved positive by calf birth, 93 (64 per cent) took place in the right horn of the uterus, and 53 (36 per cent) in the left horn.

Clark (4) made observations on cattle from 8 to 12 weeks in gestation by rectal palpation of the uterine horns, ovaries, and middle uterine arteries. He found that in 704 cases of single pregnancy 293 (42 per cent) were in the left uterine horn and 411 (58 per cent) in the right uterine horn. In none of the cases observed by Clark was there evidence of migration of the fertilized ovum into the uterine horn opposite the site of origin.

Casida, Chapman, and Rupel (5) made an extensive study of the genitalia of heifer calves. The mean weight of the right ovary from 190 calves of Holstein appearance was  $1.01 \pm .05$  grams, the mean weight of the left ovary was  $0.89 \pm .04$  of a gram. In calves of other breeds the mean weights of the ovaries were: right  $1.12 \pm .08$  grams; left,  $1.03 \pm .08$  grams. They found a significant difference in the total follicular volume of follicles 4 to 13 mm. in diameter between the right and left ovaries, the right having the greater value.

In a recent study (6), cattle pituitary glands were collected in a local slaughter house and during this time we were also given the opportunity of collecting the ovaries. Inasmuch as it had been reported that more pregnancies occurred in the right horn than occurred in the left horn it was

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thought that if a large number of ovaries were collected from sexually mature non-pregnant animals, one would find the corpus luteum of estrum present more frequently in the right ovary than in the left ovary. That is, if the cause of the difference in the number of pregnancies in the right and left uterine horn was due to a difference in the functional activity of the right and left ovary. In addition, in cases of pregnancy the corpus luteum of pregnancy could be determined. Accordingly, ovaries were collected and they were weighed and examined for the presence of the most recent corpus luteum or the largest follicle.

A summary of our records showed that the greater functional activity of the right ovary was apparent before the heifer reached sexual maturity. Of 136 sexually immature heifers the largest follicle occurred in the right ovary in 100 cases (73.5 per cent) and in the left ovary in 36 cases (26.5 per cent). The average weight of the right ovary was 2.71 grams and that of the left ovary was 2.29 grams.

The ovaries from 98 sexually mature non-pregnant heifers were collected. Of this number the right ovary contained the corpus luteum of estrum in 59 cases (60.2 per cent) and the left ovary in 39 cases (39.8 per cent). The average weight of the right ovary was 5.53 grams and that of the left ovary was 4.82 grams.

Ovaries from 59 pregnant cows were collected and of this number the corpus luteum of pregnancy was found in the right ovary in 39 cases (66.1 per cent) and in the left ovary in 20 cases (33.9 per cent). In all cases the corpus luteum of pregnancy occurred on the same side as the pregnant uterine horn. The results are summarized in Table 1.

TABLE 1  
*The weight of bovine ovaries*

GROUP	NO. OF ANIMALS REPRESENTED	PER CENT	AVERAGE WEIGHT OF RIGHT OVARY GM.	AVERAGE WEIGHT OF LEFT OVARY GM.
Ovaries from sexually immature heifers	136		2.71	2.29
Largest follicle in right ovary .....	100	73.5	2.91	2.14
Largest follicle in left ovary .....	36	26.5	2.16	2.73
Ovaries from sexually mature, non-pregnant heifers	98		5.53	4.82
Most recent corpus luteum in right ovary .....	59	60.2	6.64	3.48
Most recent corpus luteum in left ovary .....	39	39.8	4.00	6.85
Ovaries from pregnant heifers and cows	59		6.91	4.88
Corpus luteum of pregnancy in right ovary .....	39	66.1	8.09	4.32
Corpus luteum of pregnancy in left ovary .....	20	33.9	3.44	5.89

## CONCLUSION

The right ovary of cattle is functionally more active than the left ovary and this difference in functional activity accounts for the greater number of pregnancies that occur in the right uterine horn than in the left horn.

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# SOME OBSERVATIONS ON THE VITAMIN A VALUE OF BUTTER PRODUCED UNDER DROUGHT CONDITIONS<sup>1</sup>

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A few reports (1) appear in the literature on the vitamin A value of butter secured from cows on restricted rations. During the course of another investigation at the Kansas Agricultural Experiment Station samples of milk were collected for churning from the station Holstein herd whose normal feeding schedule has been considerably disturbed as a result of drought, leaving the herd without pasture during the usual pasture season. Commercial samples were also obtained from the station creamery at the same time for comparison. Other samples were available from an experimental group of grade Holsteins which had been maintained in dry lot on a restricted ration for over two years. It is the purpose of this paper to report the vitamin A value of butters produced under these unusual feeding conditions for comparison with known normal values.

## METHODS

Twenty-four hour collections of milk were made from these groups of Holstein cows and butter was churned from the composites. Sample No. 1 was produced from the experimental cows on restricted ration in dry lot. Their ration consisted of upland prairie hay (about No. 2 grade) and a grain mixture made up of equal parts of white corn, wheat bran and cottonseed meal. Samples Nos. 2 and 3 were from the station herd collected in September and October respectively at the end of the abnormally dry summer of 1936. Little pasture had been available for several months to supplement the herd ration of alfalfa hay (approximately No. 2 grade), Atlas sorgo silage, and grain mixture made up of 400 pounds yellow corn, 200 pounds bran, 220 pounds oats, 100 pounds cottonseed meal, and 50 pounds linseed oil meal.

About the time that the foregoing samples were collected, samples Nos. 4 and 5 were secured from the station creamery. These had been churned from patrons' milk and represented feeding conditions somewhat similar to those under which the station herd was maintained.

Vitamin A content of the butter was estimated by measuring the absorption spectra at 328  $m\mu$  with a quartz spectrograph (courtesy of Physics Department, Iowa State College). The amount of beta carotene was determined spectrophotometrically at 480, 470, and 455  $m\mu$  with the application

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<sup>1</sup> Contribution No. 74 from the Department of Home Economics, and Contribution No. 119 from the Department of Dairy Husbandry.

TABLE 1  
*Vitamin A value of butter produced under drought conditions*

SOURCE OF BUTTER SAMPLE	SAMPLE NO.	DATE OF COLLECTION	VITAMIN A CONTENT PER GM. 328 M $\mu$		B-CAROTENE PER GRAM AVERAGE FROM 450, 470, 485 M $\mu$		TOTAL VITAMIN A VALUE PER GRAM	FAT YIELD PER COW	AVERAGE VALUE OF DAILY YIELDS
			Average per cent	Average I.U.	Average per cent	Average I.U.			
Holstein experimental ration	1	9/9/36	.000646	10.08	.000081	1.34	11.42	gm.	1,000 I.U.
	2	9/3/36	.001053	16.43	.000211	3.50	19.93	556	13
Holstein herd ration	3	10/19/36	.000864	13.48	.000326	5.31	18.79	419	9
Commercial samples	4	8/29/36	.000978	15.26	.000269	4.46	19.72	...	...
	5	10/19/36	.000771	12.03	.000419	6.95	18.98	...	...

of Peterson's (2) extinction coefficients. Results of the summation were computed and expressed as International Units of vitamin A value per gram of butterfat (Table 1). This method of determining vitamin A value of butter has been found by Leuschen *et al.* (4) to give results which check findings secured by the single feeding biological method.

#### DISCUSSION

The total vitamin A value of butter produced by the experimental herd on the restricted ration for over two years (Table 1) was the lowest for the samples tested. It contained approximately 11 I.U. per gram, or less than one-fourth the figure for butter computed by Sherman (3). The vitamin A value contributed by beta carotene in this sample was particularly low, averaging only about 30 per cent of that in the herd samples (Nos. 2 and 3). This low figure can be attributed to the absence of green feed or pasture in the ration of these cows and the presumably lower quality of the hay consumed. The September and October samples collected from the station Holstein herd at the end of the abnormally dry summer also showed low vitamin A values, averaging only about one-half those given for butter in the Sherman tables. These cows had received very little pasture since the previous spring. Summer temperatures also were the highest on record, which may have had some influence on vitamin A metabolism. While they were fed a ration typical of good barn feeding conditions, the hay and silage were of lower quality than in more normal years. This undoubtedly was true of much of the roughage harvested over extensive areas of the country seriously affected by the drought. In the total vitamin A value the butter runs considerably below computed average values (3) representative of good feeding. The question may be raised whether under drought conditions with much of the roughage fed of poor quality, the vitamin A value of the milk and butter produced is so reduced as to call for additional safeguards in human diets. At the time the October samples were collected, the cows had access to wheat and rye pasture for short intervals over a period of 10 days. This is reflected in the more than 50 per cent increase in the percentage of carotene, though with no increase in the total vitamin A value. The explanation for this is not known. In many experiments, a close correlation has been observed between the carotene content and vitamin A value of the milk fat. This does not always appear to hold true, however, Loy *et al.* (5) have observed that under certain conditions, especially those which affect the utilization of the vitamin of the ration or interfere with the vitamin A metabolism of the cow, there may be little or no correlation between carotene content and vitamin A activity of butterfat. The commercial samples which were produced under somewhat the same feeding conditions showed similar behavior and values closely approximating those for the station herd. The vitamin A value of the average daily yield of milk per cow also was calculated

(Table 1). This shows that in terms of total production the experimental herd with the lower level of milk production had lowest total daily yield of vitamin A value, averaging less than half the yields of the herd Holsteins.

#### SUMMARY AND CONCLUSIONS

Vitamin A values, determined by physical-chemical methods, are reported for butter samples produced under drought conditions. One group of Holstein cows had been maintained in dry lot for a period of two and a half years on a ration of prairie hay (approximately No. 2 upland) and grain mixture made up of equal parts white corn, wheat bran, and cottonseed meal. Other samples were secured from the station Holstein herd after the abnormally dry summer of 1936. This herd had received a ration of alfalfa hay, sorgo silage and a grain mixture with very little pasture since the previous spring. Values are also reported for commercial samples churned about the same time in the college creamery from patrons' milk produced under somewhat similar feeding conditions.

The vitamin A value of the butter produced by the experimental herd in dry lot was approximately 11 I.U. per gram or less than one-fourth the values computed by Sherman for butter produced under good feeding conditions. The vitamin A value of the beta carotene of this sample averaged only 30 per cent of the station herd samples.

The samples from cows in the station Holstein herd following the abnormally dry summer of 1936 showed vitamin A values averaging approximately one-half normal figures.

Commercial samples secured at the same time and produced under similar feeding conditions gave comparable values.

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# THE ACTION OF HERRING OIL BEFORE AND AFTER HYDROGENATION ON THE YIELD AND FAT PERCENTAGE OF THE MILK OF THE GOAT<sup>1</sup>

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When small amounts of cod-liver oil are included in the diets of lactating cows, there is a subsequent decline in the fat percentage of the milk which they secrete. This was first pointed out by Golding and his associates (1), and has since been amply substantiated by Petersen (2) and McCay and Maynard (3).

Recently Golding (4) has shown that the factor or factors in cod-liver oil which cause this decline in fat percentage are not recovered in the non-saponifiable fraction of the oil. McCay and Maynard (3) have shown that the principle responsible for the phenomenon is located in the saponifiable fraction of the oil. In other words, some fatty acid or groups of fatty acids is responsible.

McCay and Maynard fed not only cod-liver oil but shark and salmon oil. These, however, produced an effect that was much smaller than that experienced with cod-liver oil.

In the light of these findings it would appear that the fatty acid group responsible for the reaction would be present in other fish oils but possibly in smaller quantities. The reaction, if of general biological importance, should be manifest in other species. Other highly unsaturated fatty acids will not produce a similar effect in lowering the percentage of fat in the milk. However, this does not necessarily mean that certain groupings of unsaturated bonds possibly occurring in the fatty acids of cod-liver oil are not responsible for the phenomenon. If this were true, hydrogenation of the fatty acids would obliterate the effect as produced by the untreated fish oil.

## EXPERIMENTAL

Six lactating goats were used in the experiment, which was continued over a period of 11 weeks. The animals were divided into two groups and their ration supplemented as shown in Table 1 with herring oil and the same oil after hardening by hydrogenation.

The animals were confined to their stalls throughout the experiment. The milk secreted was measured to the nearest 5 cc. and milk fats were determined

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TABLE 1  
Showing the fat supplement to the daily diet

GROUP	WEEK				
	1-3	3-5	6	6-9	9-11
1	Control	2 oz. A	2 oz. B	2 oz. B	2 oz. B
2	Control	2 oz. B	2 oz. A	2 oz. A	2 oz. B

A—Original herring oil.

B—Hydrogenated herring oil.

on the milks of each animal twice weekly from a composite sample made up by taking aliquots from each milking. The original plan of experiment called for a mixture of the fat in the diet. However, while the goats consumed their ration containing the hardened oil with their usual appetite, they refused the mixture containing the original oil. Consequently, this was fed by syringe throughout the experiments.

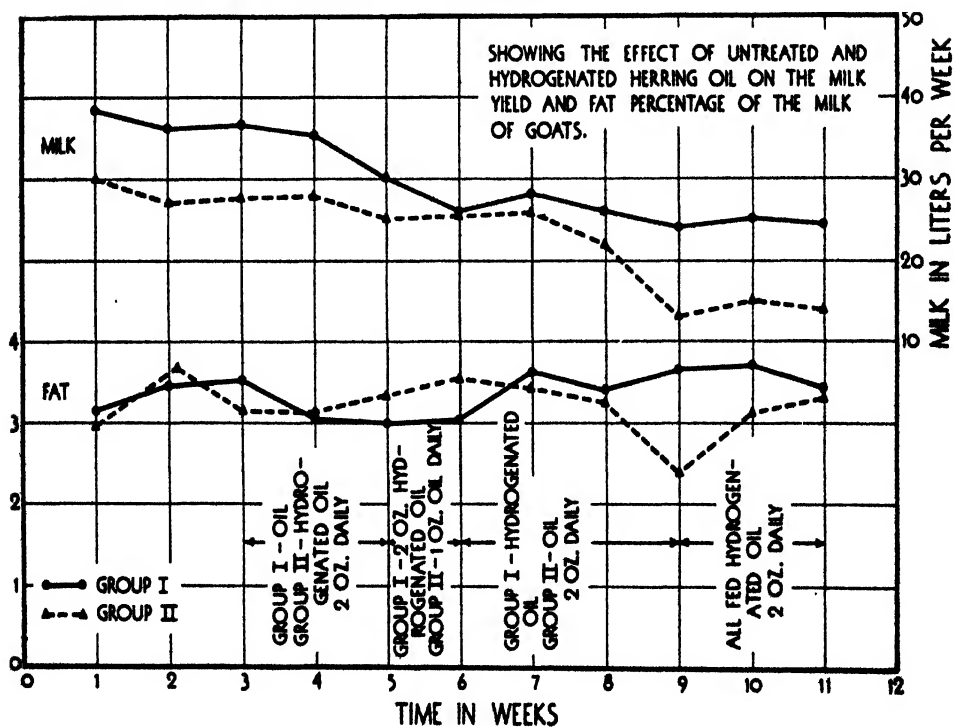


FIG. 1.

The condensed results are shown in Table 2 and Figure 1. These demonstrated that when 2 ounces of herring oil were fed daily there was a subsequent reduction in the percentage of fat in the milk produced by these animals. Similar amounts of the same fat after hydrogenation had little

TABLE 2  
Showing the condensed results for milk yield and percentage of fat

COW NO.		WEEK										
		1	2	3	4	5	6	7	8	9	10	11
Group 1												
123	Milk (cc.)	16,705	15,290	14,660	12,520	11,720	11,530	11,570	10,150	9,980	9,480	10,120
	Fat %	3.60	4.11	4.21	3.85	3.76	3.50	4.17	4.15	4.38	4.19	3.86
30	Milk	9,495	8,625	9,130	8,120	6,750	5,830	6,920	6,840	5,320	6,840	5,390
	Fat %	2.24	2.65	2.65	2.30	2.18	2.15	2.70	2.31	2.50	2.49	2.72
119	Milk	12,480	12,370	12,920	12,700	11,390	9,430	10,130	9,810	9,090	9,820	9,270
	Fat %	3.18	3.25	3.39	3.22	2.975	3.10	3.70	3.40	3.61	3.766	3.49
	Mean											
	Milk	36,860	36,285	36,710	35,340	30,860	26,970	28,620	26,700	24,390	25,140	24,780
	Fat %	3.13	3.47	3.53	3.05	3.00	3.06	3.64	3.41	3.67	3.73	3.47
Group 2												
256	Milk	9,740	8,960	9,280	9,240	8,560	9,130	9,340	7,740	7,260	8,230	4,510
	Fat %	3.16	3.99	3.15	3.45	4.139	3.85	4.10	3.06	3.37	3.59	3.81
252	Milk	10,130	9,390	9,290	9,500	9,250	8,810	9,180	8,320	4,890	3,265	5,710
	Fat %	3.48	3.62	3.86	3.46	3.284	3.60	3.08	2.39	2.93	3.83	3.27
507	Milk	10,120	8,845	9,410	9,480	8,380	8,840	8,430	6,870	1,504	3,850	4,920
	Fat %	2.3	2.76	2.60	2.59	2.626	2.90	2.35	1.66	2.60	2.47	2.31
	Mean											
	Milk	29,990	27,195	28,050	28,220	26,190	26,780	26,990	22,950	13,654	15,345	14,200
	Fat %	2.97	3.68	3.19	3.16	3.349	3.45	3.205	2.395	3.13	3.36	3.34

or no effect on the percentage of milk fat. In fact, if any change occurred, there may have been a slight increase under this condition of feeding.

The results show that hydrogenation of the herring oil destroyed its power to reduce the percentage of fat in the milk. This fact indicates that the factor or factors responsible for the reaction are in unsaturated bonds of the fish oils. Since, however, simple unsaturation has been shown to produce no similar effect, the results must be due to some particular grouping of unsaturated bonds in these fatty acids.

The treatment with herring oil was, however, not without effect on the general well-being of the animals. This did not become apparent until the animals had been on feed for over two weeks. At that time they began to refuse their feed and the decline in milk production and fat percentage manifest in the earlier portions of the period became more pronounced. No difficulties were experienced when the animals were fed the supplements of hardened fat.

The reductions shown in the volume of milk secreted when the oil was fed are thus complicated by reduced feed intake. However, under similar conditions without the fish oil in the diet we would expect, on the basis of our knowledge of fat production, that there would have been an increase in the fat percentage of the milk.

The effect produced when the oil was fed appeared to be generalized through the body rather than localized in the secretion of the mammary gland. The toxicity of the untreated oil as compared to the same oil after hydrogenation indicates that the grouping of unsaturated bonds is reacting with some mechanism which is of general importance to the animal as a whole rather than one which might be specifically related to the secretion of milk.

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## American Dairy Science Association Announcements

### TO MEMBERS OF THE AMERICAN DAIRY SCIENCE ASSOCIATION :

Each year your Association has been making progress by extending or enlarging its activities. The progress that has been made is due to the interest and support the Association has received from its individual members and it seems desirable at this time to call the members' attention to certain activities of the Association which are in need of some extra effort on the part of the members of the Association. Since the members of the Association meet only once a year, it is not always possible for the officers and directors to secure the advice and suggestions that are often badly needed in conducting the affairs of the Association; however, a listing of a few of the things that are being considered by the officers of the Association may suggest to you a means by which you can best serve your Association.

The December number of the JOURNAL contains a list of members and subscribers by states and it is hoped that the members in each of these states will check this list and either make an effort to secure others, who would be benefited by becoming members of this Association or subscribers to the JOURNAL, or inform the secretary of such prospective members.

The JOURNAL management has been able to make another improvement. The March issue of the JOURNAL will contain the production abstracts which it has not conducted before. This will, however, increase the cost of publishing, and an increase in membership or subscriptions will be especially desirable in keeping the JOURNAL on a sound financial basis.

The membership should be considering nominations for the Borden Award for 1938, which will be for work completed and published during the five-year period, ending December 31, 1937. Nominations are to be considered for the most outstanding research work in the production field—breeding, feeding, farm sanitation, or quality production, and for outstanding research in the process field, such as improvement in equipment or methods in the handling of milk or cream and the production of milk products. Anyone who is a citizen of the United States or Canada and has not reached his fortieth birthday is eligible.

A committee has been appointed to bring the by-laws and constitution of the American Dairy Science Association up to date and make revisions where it is thought desirable. Professor S. M. Salisbury, Ohio State University, Columbus, Ohio, is chairman of this committee and will welcome suggestions from the membership in regard to desirable changes of the by-laws and constitution.

Plans are now in process for the annual meeting which will be held on the campus of Ohio State University, Columbus, Ohio, June 14 to 17, 1938.

The program committee has been appointed and the chairman, Dr. T. S. Sutton of Ohio State University will appreciate suggestions from members regarding the 1938 program.

There are very few members of the Association who cannot give some valuable assistance or suggestions on one or more of the above items and by doing so will contribute to the progress and increased usefulness of the Association. I can assure you that your assistance will be greatly appreciated by the officers and chairmen of these different committees.

Yours very truly,

H. W. GREGORY, *President*

### STUDENTS' NATIONAL CONTEST IN JUDGING DAIRY PRODUCTS

Ohio State University again won highest honors in the Students' Dairy Products Judging Contest held at the Dairy Industries Exposition in New Orleans, October 25, 1937. Last year that institution gained permanent possession of the silver cup awarded the team winning first place in judging all products, having won it three times.

In the judging of ice cream, the members of the Ohio team won first, second, and third places, which is a record performance. In the judging of other products, however, teams from other institutions carried off all the silver cups and medals. The University of Nebraska won first in judging butter, South Dakota State College first in the judging of milk, and the University of Minnesota first in the judging of cheese. Teams from seventeen colleges competed.

The scholarships which are awarded to the institutions represented by the teams winning the first six places in judging all products were won by Ohio State University, Mississippi State College, Massachusetts State College, Michigan State College, University of Minnesota, and Cornell University. The scholarships are provided annually by the Dairy and Ice Cream Machinery and Supplies Association.

That Association also provides five silver cups which are awarded to the team scoring first in judging all products and the teams winning first place in the judging of butter, cheese, milk, and ice cream.

Gold, silver, and bronze medals are also awarded by that Association to the individual contestants who win first, second, and third places, respectively, in judging each product and all products.

The International Association of Milk Dealers awarded a gold medal to the winning contestant in judging milk, and the International Association of Ice Cream Manufacturers awarded a gold medal to the winning contestant in judging ice cream.

Mr. William White of the Bureau of Dairy Industry, United States Department of Agriculture, again served as Superintendent of the contest. The official judges were: butter, L. S. Edwards; cheese, H. L. Wilson; ice cream, A. C. Dahlberg; and milk, C. J. Babcock.

Following are the teams and individuals who won high standings in the contest :

## ALL PRODUCTS

<i>Teams</i>		<i>Individuals</i>	
1—Ohio State University	319.60	1—Dan C. Roahen, Ohio State University	97.20
2—Mississippi State College	324.85	2—Henry A. Lardy, South Dakota State College	102.95
3—Massachusetts State College	333.95	3—Brooks Naylor, University of Minnesota	103.05
4—Michigan State College	336.70	4—M. Putnam, Mississippi State College	103.50
5—University of Minnesota	342.50	5—William B. Graham, Massachusetts State College	104.10
6—Cornell University	348.95		

## ICE CREAM

<i>Teams</i>		<i>Individuals</i>	
1—Ohio State University	72.75	1—Dan C. Roahen, Ohio State University	21.75
2—Cornell University	94.75	2—George E. Neeley, Ohio State University	24.75
3—Michigan State College	99.25	3—J. Atlee Miller, Ohio State University	26.25
4—Massachusetts State College	107.25	4—H. Webster, Cornell University	27.25
5—Louisiana State University	116.40	4—Henry A. Lardy, South Dakota State College	27.25

## BUTTER

<i>Teams</i>		<i>Individuals</i>	
1—University of Nebraska	52.25	1—Wayne Klammm, Kansas State College	9.50
2—Mississippi State College	55.75	2—Oakley Larson, University of Nebraska	14.75
3—University of Minnesota	58.00	3—Don Radenbaugh, University of Nebraska	15.00
4—Iowa State College	58.50	4—William B. Graham, Massachusetts State College	16.00
5—Michigan State College	65.50	5—Howard G. Dissaly, Cornell University	16.50

## MILK

<i>Teams</i>		<i>Individuals</i>	
1—South Dakota State College	67.45	1—David Henry, South Dakota State College	14.15
2—Mississippi State College	70.85	2—Robert D. MacCurdy, Massachusetts State College	16.80
3—Texas Technological College	72.70	3—Clement C. Schmieg, University of Wisconsin	19.30
4—Massachusetts State College	76.20	4—George H. Fenner, Iowa State College	19.45
5—University of Wisconsin	79.00	5—M. Putnam, Mississippi State College	20.50

## CHEESE

<i>Teams</i>		<i>Individuals</i>	
1—University of Minnesota	67.50	1—Rudolph P. Zelm, University of Wisconsin	18.50
2—Mississippi State College	70.75	2—Brooks Naylor, University of Minnesota	21.00

3—Massachusetts State College . . . . .	72.00	2—William B. Graham, Massachu- setts State College . . . . .	21.00
4—Iowa State College . . . . .	80.50	4—J. B. Maury, Jr., Mississippi State College . . . . .	21.25
5—Cornell University . . . . .	80.75	5—Williams Welles, Michigan State College . . . . .	22.00
		5—Goodwin Sonstegard, University of Minnesota . . . . .	22.00

### COLLEGIATE DAIRY CATTLE JUDGING CONTEST

The Collegiate Dairy Cattle Judging Contest, held at the National Dairy Show in Columbus, Ohio, was operated under the rules approved by the committee of the American Dairy Science Association consisting of Drs. I. W. Rupel, J. R. Dice, P. M. Reaves, and J. F. Kendrick. Fifteen rings of animals were judged. Two rings of four animals each and one ring of six or more animals were selected for each breed. A committee was selected by the superintendent, Burt Oderkirk, to select the rings, and he appointed five official breed judges who worked with the entire group of coaches in placing the classes. This year a single judge for each breed graded the reasons. The judges and breed of cattle judged are as follows:

Holsteins—L. S. Gillette, Fostoria, Iowa.

Guernseys—O. G. Schaefer, Meredith Publications, New York.

Ayrshires—Jack Nesbit, Hoard's Dairyman, Ft. Atkinson, Wis.

Jerseys—Frank Astroth, S. St. Paul, Minn.

Brown Swiss—L. H. Fairchild, Allied Mills, Omaha, Nebr.

The American Jersey Cattle Club again awarded a \$400.00 scholarship to the high ranking contestant in judging Jerseys to apply on graduate work in dairy husbandry. Cups and other trophies were awarded by the breed association and commercial companies. Significant, according to the judges of the contest who had judged the contest several years ago, was the absence of poorly trained teams, which would indicate that livestock judging instruction has been strengthened in many institutions.

Following are the teams and individuals who won high standings in the contest:

#### ALL BREEDS

<i>Teams</i>		<i>Individuals</i>	
1—Texas A. & M. College . . . . .	5365.9	1—S. R. Aldrich, Michigan . . . . .	1818.4
2—Oklahoma A. & M. College . . . . .	5243.7	2—M. Underwood, Purdue . . . . .	1802.7
3—Purdue University . . . . .	5217.4	3—R. Nelson, Wisconsin . . . . .	1802.6
4—Michigan State College . . . . .	5214.1	4—J. Bradley, Texas A. & M. . . . .	1797.9
5—Kansas State College . . . . .	5191.5	5—B. B. Hodgins, Ontario . . . . .	1797.4

#### AYRSHIRES

<i>Teams</i>		<i>Individuals</i>	
1—Texas A. & M. College . . . . .	1145.6	1—J. W. Bradley, Texas A. & M. . . . .	394.6
2—University of Missouri . . . . .	1124.7	2—Warren Westbrook, Missouri . . . . .	390.4
3—University of Tennessee . . . . .	1102.9	3—N. C. Fry, Texas A. & M. . . . .	388.6
4—Pennsylvania State College . . . . .	1093.3	4—Loyol Corman, Nebraska . . . . .	384.6
5—Cornell University . . . . .	1087.1	5—Alfred Durand, Pennsylvania . . . . .	384.0

## BROWN SWISS

<i>Teams</i>		<i>Individuals</i>	
1—Michigan State College	1137.6	1—N. C. Fry, Texas A. & M.	390.7
2—Texas A. & M. College	1102.6	2—C. Weaver, Michigan	389.2
3—University of Wisconsin	1091.8	3—Wilson Jones, Tennessee	382.6
4—Purdue University	1040.5	4—S. R. Aldrich, Michigan	379.0
5—Ohio State University	1036.6	5—Robert Van Liere, Wisconsin	378.8

## GUEKNSEYS

<i>Teams</i>		<i>Individuals</i>	
1—University of Maryland	1061.1	1—Frank McFarland, Maryland	372.0
2—Oklahoma A. & M. College	1053.3	2—J. B. Outhouse, New York (Cornell)	367.4
3—Ontario Agr. College	1040.6	3—Ernest Zehner, Ohio	364.0
4—University of Missouri	1040.0	4—Loyol Corman, Nebraska	363.1
4—University of Nebraska	1040.0	5—Everett Danney, Ohio	261.9

## HOLSTEINS

<i>Teams</i>		<i>Individuals</i>	
1—Texas A. & M. College	1076.1	1—R. Nelson, Wisconsin	375.6
2—University of Wisconsin	1062.9	2—M. Jones, Texas A. & M.	373.9
3—University of Nebraska	1059.9	3—V. Brown, Iowa	372.7
4—Oklahoma A. & M. College	1059.3	4—W. Englund, Nebraska	370.6
5—University of Missouri	1043.3	5—B. B. Hodgins, Ontario	367.0
		5—B. Bennett, Oklahoma	367.0

## JERSEYS

<i>Teams</i>		<i>Individuals</i>	
1—Kansas State College	1097.1	1—Forest Fansher, Kansas	385.2
2—Texas A. & M. College	1087.3	2—R. Nelson Phelps, Maryland	378.4
3—University of Maryland	1080.8	3—Clyde Chappell, Tennessee	377.6
4—University of Tennessee	1075.8	4—S. R. Aldrich, Michigan	375.6
5—Oklahoma A. & M. College	1072.9	5—Vernon Baldwin, Minnesota	372.0

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## NUTRITIONAL ANEMIA IN CATTLE IN SOUTHEASTERN MASSACHUSETTS\*

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### INTRODUCTION

During the past few decades nutritional anemia among cattle has been identified in certain widely scattered but relatively restricted areas of the world. The disease under various names has been known to stockmen for a much longer time, but its cause and the effective treatment of it were first demonstrated by Aston (1) in New Zealand early in the present century. Since Aston published his findings the disease has been reported by Orr (2) from certain areas in Scotland and from Kenya colony in Africa, by Sjol- lema (3) from Holland, and by Becker *et al.* (4) from Florida.

The disease is variously known as "bush sickness" in New Zealand, "nakuruitis" in Kenya, "pining" or "vinquish" in Scotland, "lecksucht" in Holland, and "salt sick" in Florida. It is characterized by a general unthrifty condition, loss of appetite and consequent emaciation, and by a definite lowering of the number of erythrocytes (red cells) and of the hemoglobin in the blood of the affected animals.

In most cases an insufficient amount of iron in the soils and crops of the region has been shown to be the cause of the trouble, although at least one group of investigators has reported that the iron deficiency may be complicated by a copper deficiency (4). Generally cures have been effected by supplying iron directly to the animals as a drench (iron ammonium citrate) or by allowing them access to a mineral lick (red oxide of iron and salt).

### OCCURRENCE IN MASSACHUSETTS

Early in 1934 the attention of one of us (S. L. F.) was drawn to an unusual condition in a valuable herd of Guernsey cattle on an estate bordering on the north side of Buzzards Bay in Plymouth County. The mature cows were apparently normal, but the young stock had a decidedly unthrifty

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appearance. One heifer had died, another was apparently in the advanced stages of the trouble, while several others represented less advanced stages of varying degrees of severity. The herdsman stated that the heifers had no appetite, especially for grain, and that when the trouble had progressed to its more advanced stages he could not induce them to eat grain at all.

This peculiarity suggested a clue; since the mature cows were eating liberally of grain and were not visibly affected, some deficiency in the roughage seemed indicated. Search of the literature suggested that the trouble might possibly be an iron deficiency. Accordingly samples of the hay and corn silage that were being fed were analyzed for their iron and copper content. Calcium and phosphorus were also determined to eliminate the possibility of deficiency of those elements. As a further check on the identity of the trouble the hemoglobin content of the blood of several of the animals was determined. Results of these determinations are shown in tables 1 and 2, together with some normal values for comparison.

TABLE 1

*Mineral analyses of hay and corn silage which the affected cattle had been eating*

SAMPLE NO.	DESCRIPTION	PERCENTAGE IN THE DRY MATTER			
		Calcium	Phosphorus	Iron	Copper
1	Hay as eaten by the cattle	.33	.20	.0087	.0011
2	Hay-residue refused by the cattle	.26	.20	.0074	not determined
3	Hay from an area a few miles distant where the trouble is unknown	.69	.28	.0111	.0014
4	Hay { presumably normal from other sections of the country }	.24	.16	.0342	not reported
5		.19	.17	.0215	
6	Hay from New Zealand where similar trouble is experienced	not reported		.0065	"
	Corn silage, from the farm in question	.25	.30	.0008	not determined
	Corn silage, average analyses	.36	.20	none on record	

The principal points which these analytical results bring out are, (1) the iron content of the roughage is somewhat below that for hays from other sections of the state and of the country where such trouble is not experienced, and (2) the hemoglobin content is somewhat below normal in the blood of the animals visibly affected by the disease.

On the basis of these results a recommendation was made to the manager of the farm in question that as an emergency measure he administer to the affected animals a daily dose of readily available iron. This was given as a

TABLE 2  
*Hemoglobin content of the blood of several heifers in the affected herd*

HEIFER NO.	HEMOGLOBIN FOUND (GMS. PER 100 CC. OF BLOOD)	PERCENTAGE OF NORMAL*	REMARKS
1	9.6	79.3	In advanced stage of the disease.
2	8.8	72.7	Affected but not so far advanced as #1.
3	9.8	81.0	Showing some symptoms.
4	12.2	100.8	Apparently normal.

\* Normal = 12.1 grams of hemoglobin per 100 cc. of blood—a tentative standard obtained by averaging values for the blood of heifers as reported in a compilation by Neal and Becker representing 70 analyses (5).

Hemoglobin was determined with a Hellige colorimeter according to the method described in a booklet published by the makers of the instrument (Hellige, Inc., New York), in November, 1931. The method was developed by Wintrobe and Miller, of Johns Hopkins Hospital, Baltimore, Maryland.

drench in the form of a solution of iron ammonium citrate, one-half pound to a gallon of water,<sup>1</sup> two fluid ounces being the daily dose. As indicated in figures 1 and 2, the animals made a phenomenal recovery.

In order to prevent recurrence of the trouble it was further recommended that a change be made in the type of roughage fed. Alfalfa and grass hay

<sup>1</sup> Strength recommended by Becker *et al.*, in Florida Experiment Station Bulletin 231.



FIG. 1. HEIFER SUFFERING FROM AN ADVANCED CASE OF "NECK AIL." PHOTOGRAPH TAKEN IN MARCH, 1934. THE BLURRED WHITE SPOTS ON THE JAW, NECK AND SIDE ARE WHERE HAIR HAD COME OUT.

not grown on the farm were substituted for part of the home-grown hay and no further trouble has been reported.

Contemporaneous with the investigation of this particular case inquiries were made of farmers in the surrounding territory in an endeavor to ascertain the extent of the trouble. Specific cases of the trouble were found on two other farms, one located in an adjoining township, the other several miles distant.



FIG. 2. THE SAME HEIFER IN MAY OF THE SAME YEAR, AFTER RECEIVING A DAILY DRENCH OF IRON AMMONIUM CITRATE FOR TWO MONTHS. SHE GAVE BIRTH TO A NORMAL CALF THE FOLLOWING OCTOBER; WHEN SEEN AT THAT TIME SHE WAS IN EXCELLENT CONDITION AND WAS MILKING OVER 30 POUNDS DAILY.

Old residents of the region told of a condition formerly quite prevalent among their cattle which they termed "neck ail," the symptoms of which, judging from the description they gave, were very similar to, if not identical with the trouble described above. The name originates, not, as one might suppose on first hearing it, from any tendency of the disease to affect the necks of the cattle, but in the observation made by an earlier generation of farmers that cattle succumbed to it when confined in their grazing to, or when fed roughage grown on, the "necks" of land that run out into the bays, a common feature of the topography of the region.

It appears that among the local residents certain areas have been definitely recognized for many years as centers of the trouble. Formerly attempts to winter cattle in these areas on home-grown roughage resulted in such a high incidence of the disease that it became common practice to send

the cattle to other so-called "healthy" areas for the winter. The change either prevented the onset of the disease, or if the animals were already subject to it, a complete recovery was soon noted. Generally the "neck ail" areas occurred on shore farms, while for the most part the "healthy" areas were several miles inland.

Other expedients for combatting the trouble were also brought to light. One individual reported that it had been his practice to burn lumps of copperas (iron sulphate), grind it fine and mix it with the grain for his cattle. Another one said that having been troubled for years with "neck ail" among his cattle he recently discovered that he can control it by feeding a certain proprietary condition powder. He stated further that a neighbor uses the same powder sometimes and has no trouble, but tries to get along without it at other times only to have the disease reappear.

Since this man's experience suggested a further clue, a pound box of this particular condition powder was purchased in the open market and subjected to chemical analysis. Among other things it was found to contain 5.6 per cent of iron (equivalent to 8 per cent of red oxide of iron,  $\text{Fe}_2\text{O}_3$ ) but not more than a trace of copper. The presence of the iron oxide was evident even from superficial examination, the material being brick red in color.

A number of those interviewed emphasized the fact that the trouble was more prevalent formerly than it has been of late years. Two factors in the situation suggest an explanation for this: (1) the number of cattle in the region is much less than it used to be, and (2) more imported hay is fed.

#### ADDITIONAL ANALYTICAL STUDIES

This fund of supporting information was then supplemented by further analyses of hays and soils of the region for their iron content. A summary of the results together with appropriate comparisons appears in table 3.

In general these results show a much higher iron content in both hay and soil from farms where the trouble is unknown than from those where definite cases of "neck ail" have been found. The percentage of iron in Hay #4 was almost exactly double that found in either #2 or #5. The salt hay (#3) had over ten times as much iron as either of these two. It is possible that some of this may have been from adherent soil due to tide flowage.

The soils from the "neck ail" areas were decidedly low in total iron. Even the "healthy" soil contained only about a third as much as the average found in a number of soils of this state several years ago. The available iron (as determined by solution of the soil in 5 per cent oxalic acid) was roughly proportional to the total amount found, being of the order of 5 per cent or less of the total. The proportion of it is slightly higher in the soils from farms where no trouble occurred. Apparently there is no correlation between the availability of the iron and the pH of the soil.

TABLE 3  
*Percentages of iron in some hays and soils of the region*

SAMPLE NO.	DESCRIPTION	TOTAL IRON %	AVAILABLE IRON %	AVAILABLE IRON AS % OF TOTAL	PH OF SOILS
1	Hay—same as in Table 1	.0087			
2	Hay from another farm where "neck ail" was noted	.0107			
3	Hay—salt hay from a shore farm in the same region, free from the trouble	.1091			
4	Hay from a farm in the so-called "healthy" area a few miles inland	.0213			
5	Hay from a 3rd farm where a case of "neck ail" was found more recently	.0107			
1	Soil on which Hay #1 was grown	.608	.0224	3.7	5.65
2	Soil on which Hay #2 was grown	.246	.0098	4.0	4.65
3	Soil from same farm as Hay #3, but not the identical soil	.577	.0326	5.7	5.70
4	Soil on which Hay #4 was grown	1.048	.0504	4.8	5.60
	Average of a number of Mass. soils	2.905			

*Additional notes on soils.*

- #1. From a shore field; surface soil—dark brown sand, 8"-12" deep; subsoil—orange yellow sand; luxuriant growth of grasses.
- #2. Grey sand, 4"-6" deep; orange sandy subsoil, sorrel very thick; much yellowing of grass and clover.
- #3. Black sandy loam, almost a muck; 8"-12" deep, gray sand subsoil.
- #4. Dark brown sandy loam, 8"-12" deep; orange sandy subsoil; good grass.

As indicated in the footnotes to table 3, the two soils (1 and 2) from farms where specific cases of the disease were found are sands, while the other two (3 and 4) would be classed as sandy loams. This is in agreement with the work of Bryan and Becker (6) in Florida who report over twice as much silt and clay in samples of the surface foot of soil from the "healthy" areas as in similar samples from areas where nutritional anemia is prevalent. It may help to explain in part why the trouble is unknown on the farm from which soil #3 came. Although this soil has slightly less total iron than soil #1 has, the available iron is somewhat higher. It is believed, however, that more probable reasons for the absence of "neck ail" on this farm, located at the approximate center of the affected area, are to be found in the following facts: (1) the owner feeds considerable salt hay, which as already noted carries a lot of iron (whether extraneous iron or not probably matters not in practice), and (2) he buys considerable hay that

has been grown elsewhere. Incidentally impartial outsiders who know the region rate him as one of its best farmers.

Reviewing all the foregoing evidence—the outward symptoms of the disease, the lowered hemoglobin content of the blood of the affected cattle, the comparatively low iron content together with an apparently adequate copper content of the hay they had been eating, the phenomenal recovery of the animals when an iron compound was administered, the experience of old residents and their methods of controlling the trouble, the low total iron content of the soils from affected farms, and the much higher iron content of soils from unaffected regions—it seems reasonable to conclude that the disease locally termed “neck ail” is identical with the nutritional anemia described under various names from other parts of the world; that it is caused by iron deficiency in the roughage, uncomplicated by copper deficiency as is sometimes the case; and that this in turn is due to a low content of iron in the soils on which the roughage is grown.

#### FURTHER SOIL STUDIES

Having reached the above conclusion the next logical step was to ascertain whether the iron content of the roughage grown on such soils could be increased by the addition of iron compounds to the soil.

Bulk samples of the soils mentioned in table 3 were secured and with them a series of pot experiments was set up under controlled conditions at the experiment station in Amherst<sup>2</sup> according to the following plan.

- Soil #1—Pot A—Check—no fertilizer.  
                   Pot B—Complete fertilizer.  
                   Pot C—Complete fertilizer plus iron.

The same arrangement for all four soils replicated once made a total of twenty-four pots. The fertilizer materials used and rates per pot were:

Sodium nitrate	1.52 grams.
Ammonium sulphate	1.18 grams.
Mono-calcium phosphate	.89 gram.
Potassium sulphate	.92 gram.

These amounts furnished acre equivalents of 200 lbs. each of nitrogen (N), phosphoric acid ( $P_2O_5$ ), and potash ( $K_2O$ ). All chemicals used were Merck's C.P. grade to obviate possibility of introducing traces of iron. To those pots scheduled for an addition of iron it was supplied in the form of ferric ammonium citrate, 1.46 grams per plot. This was the equivalent of 100 lbs. of elemental iron (Fe) per acre. The soil in the pots was kept at a uniform moisture content by the daily addition of weighed amounts of distilled water in accordance with their moisture-holding capacity.

<sup>2</sup> Acknowledgment is made of the cooperation of the Department of Agronomy of the Massachusetts State College, whose greenhouse and laboratory facilities were utilized for this phase of the work.

The crop grown was a mixture of timothy and red top, an approximately equal number of seeds of each species being used. The seeding was done in the autumn; because of the shortness of the days, growth was slow until the following spring. Probably this was not a disadvantage as it is similar to the procedure followed in this state, of seeding grass for mowings in August or September. Probably because of thickness of stand, the plants did not reach the flowering stage and were harvested the latter part of June.

Average yields are reported in table 4.

TABLE 4  
*Calculated acre yields of air-dried grass from treated soils*

SOIL*	CHECK (TONS PER ACRE)	COMPLETE FERTILIZER (TONS PER ACRE)	COMPLETE FERTILIZER + IRON (TONS PER ACRE)
#1 . . . . .	1.7	3.8	3.1
#2 . . . . .	2.3	5.3	5.7
#3 . . . . .	1.4	5.9	5.7
#4 . . . . .	2.3	6.0	5.9

\* See footnote, table 3, for identification.

The yields in table 4 show a decided response to the use of complete fertilizer either with or without iron. This is not surprising on soils as sandy in nature as these are, especially in view of the fact that the soil samples were obtained in October, at which time of the year much of the plant food applied the previous spring would have been removed either by the growing crop or by leaching.

In general the addition of iron ammonium citrate resulted in a small decrease in yield when compared with complete fertilizer without the addition of iron, but with the exception of the results for soil #1 the difference is so slight as to be within the limit of error of this type of work. It should be borne in mind, however, that iron forms insoluble compounds with phosphorus and this fact may account for the reduction in yield which occurred when the iron supplement was added. In the case of soil #2 there was a slight further increase in yield when iron was added. Incidentally this soil had much the lowest amounts of both total and available iron of any in the group (see table 3).

Of greater interest than the yields of grass are the percentages of iron resulting from the soil treatments. These appear in table 5.

The use of a complete fertilizer brought about only a small average increase over the check in the percentage of iron in the grass (less than 5 per cent.; in fact in two cases (soils 1 and 4) there was a decrease in the amount of iron in comparison with the control. The addition of an iron compound resulted in a uniform and consistent large increase in the iron content of the grass, the average being almost 58 per cent. The average

TABLE 5

*Percentages of iron in air-dry grasses after application of soil amendments*

SOIL	WITHOUT TREATMENT	WITH COMPLETE FERTILIZER	WITH COMPLETE FERTILIZER PLUS IRON	PERCENTAGE INCREASE IN IRON	
				Over check	Over complete fertilizer
	%	%	%	%	%
#1	.0257	.0239	.0394	53.3	64.9
#2	.0309	.0404	.0492	59.2	21.8
#3	.0232	.0254	.0369	59.1	45.3
#4	.0294	.0248	.0468	59.2	88.7
Average	.0273	.0286	.0431	57.9	50.7
Average percentage increase over check		4.8	57.9		

amount of iron in the controls was .0273 per cent which increased to .0431 per cent when iron was supplied as iron ammonium citrate.

When compared with the values for iron in hay grown on these soils as reported in table 3, the values for the controls are seen to be much higher. The discrepancy, however, is only apparent; the values in table 5 are for relatively immature grasses grown under ideal conditions, while those in table 3 are for mature hays, at least one of which was overripe and woody when cut. Also the general conditions under which the two sets of values were obtained are not at all comparable.

The important point which these values show is that the addition of iron to these iron-deficient soils has resulted in a marked increase in the iron content of vegetation grown on them, and as seen in table 6, has greatly increased the supply of iron available for future crops.

TABLE 6

*Residual amounts of available iron in the soils after the grass was harvested*

SOIL	WITHOUT TREATMENT	WITH COMPLETE FERTILIZER	WITH COMPLETE FERTILIZER PLUS IRON	PERCENTAGE INCREASE IN IRON OVER CHECK
	%	%	%	%
#1	.0215	.0844	.1076	400.5
#2	.0164	.0235	.1765	976.2
#3	.0365	.0437	.1554	325.8
#4	.0598	.0738	.2039	241.0
Average	.0336	.0575	.1609	378.9
Average percent- age increase over check		71.1	378.9	

It would seem, therefore, that the addition of iron compounds to the soil of regions in Massachusetts where nutritional anemia occurs, offers an alternative method in the treatment of this disease—a method that in the long run probably would be cheaper and more practicable than direct dosage of the affected animals with iron or the purchase of feed from unaffected areas.

#### SUMMARY

A disease of cattle locally known as “neck ail,” of long standing in certain localities of southeastern Massachusetts, has been described and shown to be identical with nutritional anemia of cattle occurring in various widely scattered portions of the world and known by various names.

The disease is characterized by emaciation, loss of appetite, and a diminution in the red blood cells and in the hemoglobin content of the blood of affected animals.

It is caused by an insufficient amount of iron in the native forage which in turn is due to a very low content of iron in the soils on which the forage is grown.

As with cases reported by other investigators, spectacular recovery has followed the administration of iron compounds to the affected animals.

Addition of an iron compound (iron ammonium citrate) to soils from farms where the disease had occurred, resulted in a uniform large increase in the percentage of iron in grasses grown on these soils. This suggests an alternative method for prevention of the disease.

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NOTE: After this work had been completed and while the data were being prepared for publication, there came to our attention the recent work of Denham in New Zealand and of Filmer and Underwood in Australia. These investigators have shown that it is a lack of minute traces of cobalt that causes the particular type of nutritional anemia known in those countries as “bush sickness.” //

# THE EFFECT OF THE RIPENING PROCESS ON THE VITAMIN A CONTENT OF CHEDDAR CHEESE\*†

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Various types of cheese have long been recognized as valuable adjuvants to the human dietary. Among the several types of cheese used for human consumption, the whole milk cheeses merit special consideration. This is because these cheeses, in addition to being excellent sources of certain minerals and proteins of high biological value, are rich in milk fat. This is especially true of the whole milk cheeses.

Those cheeses which contain relatively large percentages of milk fat might be expected to be rich sources of vitamin A. In fact, a few data have appeared in scientific literature (Cook and Axtmayer (1), Coward and Morgan (2)) which indicate that the Cheddar cheese is an excellent source of this vitamin. There appear to be no data available, however, concerning the relationship of the vitamin A content of the fresh cheese curds to the vitamin A content of the marketable product. Nutritional investigators, working along other lines, have reported that the actions of certain enzymes and bacteria are definitely destructive to carotene and to vitamin A. From such reports it might appear that the vitamin A content of the ripened cheese is only a fraction of the vitamin A content of the products incorporated into the cheese. Since different types of cheese, as well as different batches of the same type, are cured for varying periods of time, depending upon various factors, it would appear that those cheeses which had been subjected to the longer periods of curing and storage should have undergone the greatest depreciation in their vitamin A content. In such cases, the rate and extent of vitamin A deterioration should undoubtedly depend upon a number of factors.

Since several types of cheese are known to contain appreciable quantities of vitamin A, and since no data were available concerning the stability of the vitamin A of cheese during the ripening process, it was believed that a study concerning the changes in the vitamin A content of Cheddar cheese during the ripening process would yield data of value.

## EXPERIMENTAL

The investigation herein reported was restricted to a study of the stability of the vitamin A of Cheddar cheese during the ripening process. The cheese

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used in these studies was a portion of a regular batch of Cheddar cheese which had been manufactured in the usual manner by the Department of Dairy Husbandry. This cheese was made from the regular run of raw milk which had been produced during the early days of March. A representative sample of cheese was taken for vitamin A assay at the time the cheese was placed in the ripening chamber and similar samples were taken at intervals during the ripening process. The moisture content of each sample of cheese was determined just previous to the beginning of the assay. During the period of assay, the cheese was maintained in an air-tight container which in turn was kept in an electric refrigerator.

The vitamin A assays were carried out according to the U. S. P. technic (3) with the exception that the assay period was extended to 35 days. Each test animal was maintained at all times in an individual metal cage and was given the usual care and attention indicated by the above technic. The assay groups consisted of from six to twelve carefully selected test animals. Extreme care was exercised in distributing litters, sexes and body-weights uniformly throughout the several assay groups.

Preliminary studies with the original cheese curds at the beginning of the ripening period indicated that daily doses ranging from 0.1 to 0.4 gram were sufficient to insure satisfactory growth and at the same time made sufficient allowance for marked destruction of vitamin A during a prolonged ripening process. Therefore, this range of daily allotments was adopted. Carefully weighed quantities of cheese were fed, in separate containers, to vitamin A deficient animals. While the assays were in progress, a comparable group of animals was being fed a definite unitage of vitamin A in the form of the U. S. P. reference cod-liver oil. It may be stated in connection with the weighing of the daily portions of cheese that these portions were always taken from a freshly cut surface in order to minimize the effect of moisture losses during the assay period.

#### DATA

Data obtained as the result of this series of assays have been condensed and are presented in the following graphs (Figure 1).

#### DISCUSSION

From the data presented in the above graphs it appears quite evident that the vitamin A content of Cheddar cheese does not change appreciably during a combined ripening and storage period of one year. A period of this duration is somewhat longer than that commonly used in practical cheese-making. It appears safe, therefore, to conclude that there is no serious loss in the vitamin A potency of such cheese during the usual curing period.

While the data, as presented above, indicate only a slight decrease in the vitamin A potency of the cheese as a result of the ripening process, this decrease becomes somewhat more significant when the growth responses are adjusted for the difference in moisture content of the various cheese samples

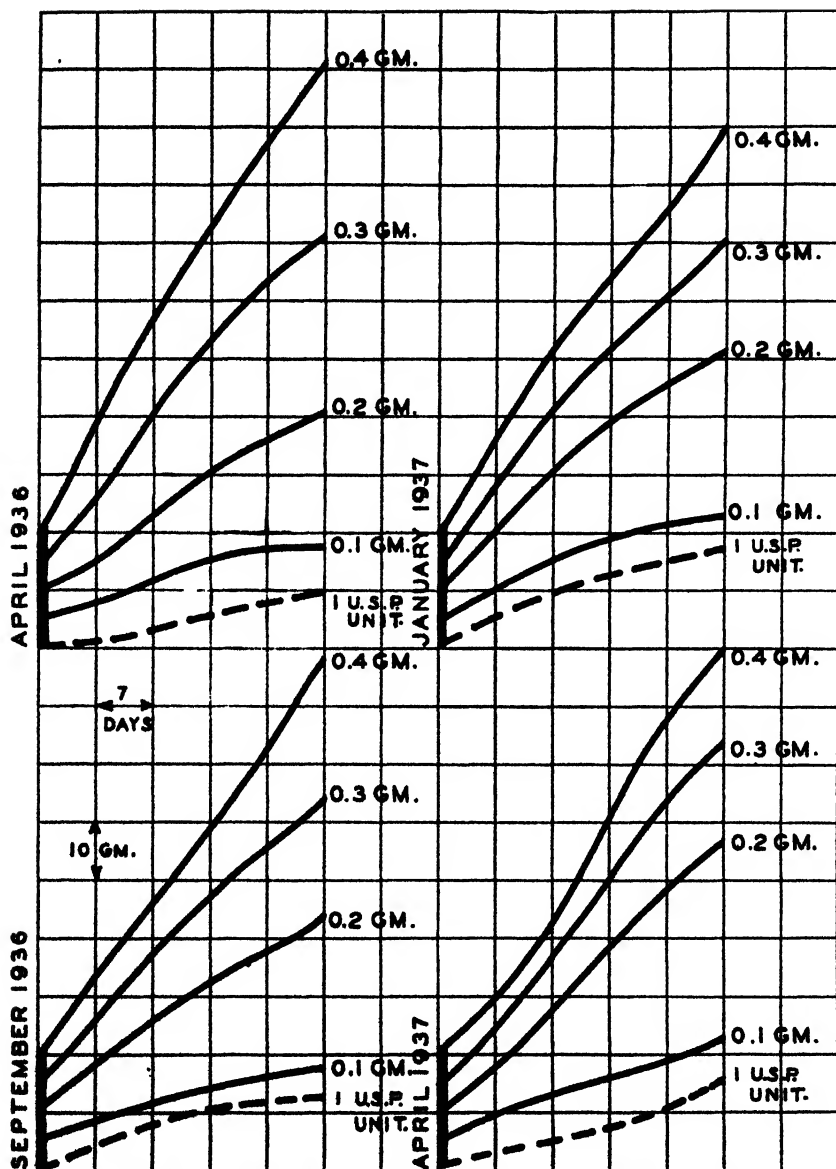


FIG. 1. Growth responses resulting from feeding definite weights of Cheddar cheese, which had been ripened for different periods of time, to groups of vitamin A deficient rats as the only supplement to a vitamin A deficient diet, are given. Corresponding growth responses resulting from feeding a definite unitage of vitamin A in the form of the U. S. P. reference cod-liver oil are also given.

at the time of assay. The moisture content of the four samples of cheese at the beginning of the assay was 37.36, 33.25, 30.29 and 30.00 per cent, respectively. Owing to moisture losses, the amounts of moisture-free cheese fed

at the second and subsequent assays were larger than those fed during the initial assay and, therefore, should have produced greater growth responses if there had been no vitamin A destruction. Since the growth responses obtained in the above studies were somewhat less, in spite of the slight increase in the amounts of moisture-free cheese fed, it can only be concluded that there had been a measurable amount of vitamin A destruction during the ripening process. But since these losses in vitamin A potency were so nearly compensated for by the decrease in moisture content of the cheese, the loss in vitamin A units per unit weight of cheese to the purchaser, as a result of the ripening process, appeared to be insignificant.

According to the above data, the finished cheese contained approximately 4500 U. S. P. units of vitamin A per pound. This potency is somewhat less than that reported for Cheddar cheese by other investigators. This difference in potency is believed to be due to differences in vitamin A intake of the cows producing the milk from which the cheese was made. It has long been known that the vitamin A content of milk (milk fat) depends upon the vitamin A intake of the cow producing it. The cheese used in these studies was made from milk which had been produced during the month of March, at a time when the vitamin A intake of the cow is relatively low as compared to other seasons of the year when green pasture, green feeds, etc., are available. The low vitamin A potency of the above cheese is, therefore, attributed to the low vitamin A content of the curds from which the cheese was made. When one considers the fact that cheese is often made from surplus milk and that surplus milk is usually more plentiful during the spring and summer months, it at once becomes apparent that much of the Cheddar cheese, as purchased on the market, may contain a vitamin A potency in excess of that reported above.

#### SUMMARY

Data are presented to show that no serious destruction of vitamin A occurs during the ripening of Cheddar cheese. While a measurable decrease in vitamin A potency was apparent, this loss was almost wholly compensated for by moisture losses. It appears, therefore, that a pound of Cheddar cheese purchased at the end of the usual ripening period will contain approximately the same amount of vitamin A as a pound of the original curds in spite of the fact that some destruction of the vitamin has occurred.

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# REACTIONS OF THE DAIRY COW TO CHANGES IN ENVIRONMENTAL TEMPERATURE

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It has been shown (1, 2, 3) that the dairy cow withstands long periods of exposure to temperatures as low as zero degrees Fahrenheit with little loss either in production or in the efficiency of food utilization. Temperatures in excess of 85° F. have, however, a marked detrimental effect (4). Because the researches from which these conclusions have been drawn were designed primarily to study the effect of exposure on milk production, and because they were subject to the many uncontrolled factors that necessarily accompany group experiments under conditions of practical dairy herd management, they yield little information of a fundamental nature that can be used to explain the physiological reactions responsible for the effects noted.

The installation at the California Experiment Station of an air conditioned room which is large enough to house two mature cows and in which temperature, humidity and air movement can be accurately controlled gave us facilities for a more precise consideration of some of the physiological reactions of high-producing cows to changes in psychrometric conditions. Six pairs of animals, including representatives of the Holstein, Jersey, and Guernsey breeds have been studied under varying conditions for periods of from three to five months. The rectal temperature, pulse, and respiration rate, together with the amount of feed and water consumed, were recorded twice daily for each animal. The physico-chemical properties of the milk were also determined. Except for changes in environmental temperature, uniform conditions were established. An air velocity of 50 feet per minute as measured by the kata thermometer and a relative humidity of 60 per cent were maintained. All cows were given a standard diet fed in accordance with their individual needs. Free access to drinking water was provided at all times.

The cows were held at each temperature for a period of from 5 to 10 days. The data for the first 2 days of each period were, however, not included in the averages, thus eliminating the possibility of any influence being exerted by the condition of the previous period. It will be seen, Table 1, that as the room temperature increases, there is a rise in the respiration rate and a fall in the pulse rate; the rectal temperature, however, remains constant at 101° F., until a room temperature of 70° F. is reached, after which it also rises.

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TABLE 1  
*Effect on respiration rate, body temperature and pulse rate of varying environmental temperatures*

ROOM TEMP.* ° F.	RESPIRATIONS PER MIN.	RECTAL TEMP. ° F.	PULSE RATE BEATS PER MIN.
40	12	101.0	....
50	17	101.0	72
60	28	101.0	68
70	42	101.3	63
80	56	101.8	61
85	70	102.2	59
90	88	102.7	60
95	106	103.7	57
100	124	105.1	

\* Air velocity at 50 feet per minute.

Relative humidity constant at 60 per cent.

Not recorded.

As shown by Table 2, the changes in the amount of milk and in its composition and chemical and physical characteristics become marked and the trends definite as the room temperature rises above 80° F. There is a decrease in milk flow, in the percentage of solids-not-fat and in the protein content. There is a lowering of the freezing point depression and a lengthening of the time of rennet coagulation. The values for the percentage of butterfat and the pH tend, however, to increase. Between 90 and 95° F. there are marked changes in the characteristics of the milk fat, as indicated by a lowering of the Reichert-Meissl value and an increase in the iodine number.

#### DISCUSSION

The normal body temperature of the cow lies between 101 and 102° F. In order to maintain it within these limits, the heat produced by metabolism must equal that lost from the body. While there is some variation in the metabolic rate and, consequently, in the rate of heat production within the body in response to thermal conditions of the environment, the balance between heat production and heat loss necessary to maintain a constant body temperature is accomplished mainly through adjusting the rate of heat loss. Our data indicate that the upper limit of heat regulation for the producing dairy cow is between 80 and 85° F., depending upon the breed. When held above this temperature for more than 24 hours, heat production overbalances heat loss, the body temperature rises, and definite changes occur in the characteristics of the milk.

The heat-regulating mechanism of the cow differs markedly from that of man in several important respects. (1) Man's principal means of rapidly increasing heat loss in hot weather is through the cooling effect of the evaporation of sweat, a method not available to the cow since, except for certain limited areas, her skin contains no functional sweat glands. (2) As shown above, the cow's respiration rate and consequent elimination of heat through

TABLE 2  
*The influence of temperature of the environment on the physico-chemical properties of milk and milk fat*

TEMP. ° F.	MILK PRODUCTION LBS. PER COW PER DAY	FAT %	SOLIDS- NOT FAT %	CASEIN %	FREEZING POINT -° C.	PH	RENNIN COAGULATION MIN. SEC.	MILK FAT CONSTANTS	
								REICHERT- MEISSL VALUE	IODINE NO.
40 ...	29	4.2	8.26	2.26	0.536	6.53	4 10	28.80	30.51
50 ...	28	4.2	8.26	2.23			3 50	29.36	31.53
60 ...	27	4.2	8.06	2.08			4 09	28.16	
70 ...	27	4.1	8.12	2.05	0.538	6.56	4 13	28.73	31.96
80 ...	25	4.0	7.88	2.07			4 00	29.14	31.77
85 ...	23	3.9	7.68	1.93			4 38	28.58	31.34
90 ...	20	4.0	7.64	1.91			4 49	28.15	31.44
95 ..	17	4.3	7.58	1.81	0.525	6.65	5 05	25.65	37.12

.. Not recorded.

the lungs increase rapidly as environmental temperature rises. By contrast, man's respiration rate, except under extreme thermal conditions, is constant. (3) While at high temperatures there is a marked acceleration of man's pulse rate, there is a slight decrease in the pulse rate of the cow.

The principal mechanism for eliminating heat from the human body, especially in hot weather, is first an increased circulation in the peripheral blood vessels, raising the temperature of the skin and thus hastening the loss of heat from it. Then, if this does not result in rapid enough cooling, perspiration begins and further loss results from the evaporation of water. There is also evidence that, at this time, there is some reduction in the amount of internal heat produced. Under conditions of high temperature, however, the regulating mechanism may prove inadequate. A rise in body temperature and a consequent speeding-up of the metabolic rate then result.

Paramount among the environmental factors governing the loss of heat from the healthy human body are the temperature, humidity and velocity of the surrounding air. For the comfort of the cow, however, humidity and air movement (except as she creates air movement through breathing) are relatively unimportant.

In order to gain a better understanding of the nature of the cow's heat regulating mechanism, we carried on an experiment to determine whether or not water was transpired through the skin. Inverted petri dishes, in the bottoms of which were fastened filter papers impregnated with calcium chloride, were taped on the rump and loin of two Jersey cows and the moisture transpired by a given area (35.7 sq. cm.) during an hour was determined. From these results it was calculated that at 84° F. and 60 per cent relative humidity, each cow transpired from her entire body surface about 1 pound of water per hour. In order to determine if this moisture resulted from sweat gland activity, the cows were thoroughly washed with distilled water, after which the chamber temperature was raised to 100° F. for 1 hour. The cows were again washed. Since this wash water gave a negative test for chlorides, we concluded that no sweating had taken place.

Our data on the relation of air temperature to the respiration rate of the cow present a very interesting study. It had been shown by others that van't Hoff's law (speed of chemical reaction approximately doubles with each increase of 18° F.) applies to the breathing rate of poikilothermous animals. Our figures for the cow fit this law remarkably well. This automatic control of temperature by breathing rate probably comprises the cow's principal method of heat regulation.

In a study of the heat dissipating mechanisms of the dog and man, Dill (7) concludes that on the whole man's is more efficient at high temperatures. In certain respects, however, he found the dog's to be superior. Since the dog and the cow have similar heat regulating mechanisms it is possible that

the points in which the dog excels may also apply to the cow. These are: (1) the absence of sweating, thus avoiding the disturbance of the water balance that accompanies the rapid elimination of salt from the body; (2) the ability to maintain an arterial carbon dioxide pressure as low as 10 mm., permitting greater alveolar ventilation, allowing for a greater dissipation of heat through the lungs, and thus avoiding the shift in circulation to the periphery which immobilizes part of the blood for gas transport; (3) the establishment of rapid air movement over significant areas through increased breathing rate.

The effect on the pulse rate is another physiological difference between man and the cow in their response to heat. While the pulse beat of the former speeds up as the "effective temperature" rises above 95° F., that of the latter tends to slow down. These facts are in thorough accord with the method of heat dissipation used by each. As stated above, man's first line of defense against a pyrexial condition is to increase the flow of blood through the capillaries of the skin; hence, an acceleration of the heart rate facilitates the elimination of heat from the body. Since the cow does not sweat, her dry skin in a hot environment may actually acquire a temperature above that of the body, in which case increasing the rate of circulation would tend to place an added burden upon the heat-dissipative mechanism while a slowing of the heart rate would be actually beneficial.

Until the cow becomes hyperthermic, her milk is quite uniform in composition and behavior. Significant changes occur in the milk after the "upper critical temperature" has been reached. These can probably be explained best on the basis of blood changes brought about to facilitate heat dissipation (5, 6, 7). The lowering of the serum protein of the blood which Dill has shown for dogs might well explain the reduced protein content of the milk of cows. The higher freezing point indicates a decrease in the soluble components of the milk. Since the cow does not sweat, the blood serum chlorides show little or no variation with increasing temperatures. This probably explains why there is so little change in the specific conductance of the fat-free portion of her milk secreted at the various temperatures. It would seem probable, therefore, that the change in freezing point noted is due to a lowering of the milk sugar content. This agrees with the observation of Lee and Scott (8) that a hot environment results in the lowering of the dog's blood sugar.

The increase in the pH of milk secreted by cows in a hot environment may best be accounted for by the decrease in the milk colloids content as affecting membrane equilibria and also by the belief of Haggard (9) that the hydrogen ion concentration of blood is decreased when the body becomes overheated. That the time of coagulation of the milk by rennin is invariably greater at the higher environmental temperatures may be partially explained

by the increased pH, although lowered calcium ion concentration may be involved.

The changes in the characteristics of the butterfat secreted at high temperatures may best be explained as the result of "hyperthermic under-nutrition." In our studies anorexia invariably accompanies the onset of the pyrexial condition and the changes in the chemistry of the butterfat conform to those found by Eckles and Palmer (10). The latter authors point out that all types of underfeeding have a marked effect on the physical and chemical constants of the butterfat, including a decline in volatile acids and an increase in the olein content.

#### SUMMARY

The effect of environmental temperature on high producing dairy cows was studied in a large psychrometric room in which the temperature was increased from 40 to 100° F., while air movement and relative humidity were maintained at the constant values of 50 feet per minute and 60 per cent respectively. It was found that, as the room temperature was increased, there was a uniform increase in the respiration rate, which approximately doubled for each increment of 18° F.; that there was a decrease in pulse rate; and that at 80 or 85° F., depending upon the breed, a pyrexial point was reached where the animals were no longer able to maintain heat balance. As the room temperature was elevated above this pyrexial point, anorexia developed, milk flow declined, and an alteration occurred in the characteristics of the milk produced, which included a lowering of the casein and solids-not-fat content, and an increase in the percentage of butterfat. The pH of the milk was raised, the freezing point depression lowered, and a longer rennet coagulation time was required. The butterfat secreted was lower in volatile acids and higher in unsaturated components.

The cow's principal avenue for heat dissipation is the respiratory system. Her breathing varies directly with the environmental temperature, and her skin has a high insulating value. She may, therefore, adapt herself with comfort to conditions of extreme cold; a fact that helps to explain why, as found by certain research workers, exposure to zero weather brings about neither lowered production nor increased maintenance requirement. It is also evident that the cow is not especially well fitted to withstand hot climates, though our studies show a distinct breed difference in this regard. Because the cow's heat regulating mechanism functions in such a radically different manner from that of man, we are not justified in applying the comfort standards worked out for man to the management of cattle. The changes in composition and physico-chemical characteristics of milk when a state of positive heat balance is reached probably result from blood changes instituted to facilitate heat disposal.

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## FLUORINE STORAGE IN CATTLE BONES

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Fluorine ingested by the animal accumulates in the bones and teeth. Michaelis (7) has postulated that fluorine plays a part in normal calcification. However, no definite proof has been advanced as to its being essential for any physiological process, even though it is always present in bones and teeth.

Recent evidence suggests that fluorine passes through the placental membrane. This is indicated by the occasional occurrence of mottled enamel in the temporary teeth of children. Smith and Smith (10), Velu (11), and Brench and Roholm (2) have reported such cases. Under experimental conditions, by the administrations of large amounts of fluorides, fluorine has been shown to accumulate in fetus of the rat and dog (8, 6).

The question of influencing the fluorine content of milk by fluorine administration is still unsettled. Murray (8) found a passage of fluorine from the mother rat to her young through the milk, but Constantini (4) found only a minute amount in the milk of goats with subacute fluorine poisoning. Phillips, Hart, and Bohstedt (9) found little change in the fluorine in cow's milk when the cows received small additions of fluorides. Huffman (5) obtained increases in the fluorine content of cow's milk when large quantities of fluorides were fed.

### EXPERIMENTAL

Bones were obtained from seven calves killed for veal and from twenty-two fetuses of different ages taken from cows which were slaughtered for beef. These were obtained from a local packing plant at Madison, Wisconsin.

The fluorine intake of the mothers of these calves and fetuses was unknown, but it was assumed to be variable. Bones from two cows which had been raised on a ration relatively low in fluorine were obtained from the University of Wisconsin herd. Two samples of commercial steamed bone meal were also analyzed. One of these was an ordinary bone meal, while the other was a specially prepared bone meal for use in baby foods.

These samples were all analyzed for fluorine by the micro-method of Armstrong (1) which is a refinement of the thorium nitrate titration method of Willard and Winter. The ash content of the bones was determined in the usual manner.

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## RESULTS

The results of the analyses as given in Table I show a fluorine content of

TABLE I  
*F content of the bones of cattle during the developmental period expressed in parts per million*

ESTIMATED AGE OF FETUS OR CALF	NO. OF ANIMALS	F RANGE	AVE. F	PER CENT ASH	F RANGE ON BASIS OF ASH	AVE. F OF BONE ASH
Embryo stage (2 to 10 weeks) . . . . .	8	18-60	37	40	45-150	92
4 to 9 months . . . . .	14	14-50	30	48	30-111	64
Day old calf . . . . .	1		24	55		44
Veal calves . . . . .	6	25-51	32	58	41-90	55
Cows (mature) . . . . .	2	177-202	190	64	278-317	298
Bone meal . . . . .	2	411-509	460	81	540-587	564

18 to 60 p.p.m. with an average of 37 p.p.m. in embryonic bone (2 to 10 weeks of age) and older fetuses had a bone fluorine content of 14 to 50 p.p.m. with an average of 30 p.p.m. The veal calf bones contained an average of 32 p.p.m. fluorine. Thus it appears that the bones of immature and mature calves are similar in their fluorine content. It seems clear from these data that the storage of fluorine in the bones of young calves remains quite low during the suckling period and further indicates a minimum secretion of fluorine in the milk. The bones from the mature cows contained an average of 190 p.p.m. fluorine, which is six times as much as those of the calves during the suckling period.

Commercial bone meal had an average of 460 p.p.m. fluorine or 564 p.p.m. on a basis of the bone ash. This is about ten times as much as the veal and fetus bones excluding embryonic bone.

Inspection of the data presented in Table I shows that embryonic bone contained appreciably larger quantities of fluorine per unit of bone ash than fetus bones. This suggested that fluorine might be present in fetal cartilage prior to calcification. With this in mind, a sample of cartilage taken from several embryos and fetuses was analyzed for its fluorine content. On the basis of dry weight this yielded 10 p.p.m. of fluorine. Since fetal bones contain on the average 30 p.p.m. of fluorine it would seem that about one-third of the fluorine content of fetal bone originates from cartilage. The ash content of this cartilage was 10 per cent and represented 25 per cent of the ash deposited in embryonic bone. The fluorine content of the fetal cartilage ash was nearly double that found in the bones of a calf at birth. A sample of whole mature bone (cow) was extracted with 10 per cent HCl until the ash was lowered to 0.4 per cent. The residue, presumably the bone matrix, was then analyzed for fluorine. It was found that this residue contained 4 p.p.m. of fluorine. Extraction with HCl had removed the greater part of the total bone fluoride along with other bone minerals from

the residue. The ash of the residue had a fluorine content of 1,000 p.p.m. Apparently the amount of fluorine in the ash of the bone matrix was considerably higher than that found in that portion of the bone soluble in 10 per cent HCl. This was 2 to 3 times the quantity expected in a normal bone ash. The significance and relationship of these results is not known. They suggest that fluorine is either a constituent of the bone matrix or that fluorine is preferentially deposited early in the calcification process.

#### DISCUSSION

Chang, Phillips, Hart, and Bohstedt (3) found an average of 584 p.p.m. fluorine in the bones of three cows which they had raised on a ration relatively low in fluorine. The additions of fluorine to the ration markedly increased the fluorine in the bones. This is much higher than what we found in the cows from the University farm, but compares favorably with our analysis of bone meals.

The relatively low fluorine content of fetus and calf bones shows that under ordinary conditions there is some passage of fluorine from the mother to the fetus through the placental membrane, and also to the calf through the milk. That the passage through the placental membrane is not the same for all cows is demonstrated by the difference in fluorine content of the bones of different fetuses of the same age groups. This may either be due to differences of permeability of the membrane or to a difference in the level of fluorine intake of the mother.

The fluorine content of the calf bones indicates that small quantities of fluorine are obtained through the milk. The amount obtained may be influenced by individual variations of the mothers.

The chief storage of fluorine seems to take place after the weaning of the calf even when it is kept on a ration containing relatively little fluorine. This is shown by the 190 p.p.m. average fluorine content of the bones of the cows on a low fluorine ration contrasted to the 32 p.p.m. average of the veal calf bones. These data fall into an interesting series of ratios. If the approximate per cent ash to fluorine in p.p.m. is taken it gives a ratio of 1:1 in the case of embryonic bone, 2:1 in fetal and veal bone and 1:3 in the case of mature cattle.

#### SUMMARY AND CONCLUSIONS

1. Under ordinary conditions it appears that a small amount of fluorine is transferred through the placenta of the cow to the fetus.

2. As long as the main source of food for the calves is milk, their bones contain a relatively constant low quantity of fluorine. Apparently small quantities of fluorine are obtained through the milk.

3. The chief storage of fluorine in the bones of cattle occurs after weaning.

4. These data also suggest that fluorine is present in appreciable quantities in the organic matrix of the bone and in cartilage.

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# RAPID METHOD FOR THE QUANTITATIVE DETERMINATION OF REDUCED ASCORBIC ACID IN MILK

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Even before the isolation and establishment of the chemical constitution of vitamin C, Tillmans (9) and his associates (10) in a series of studies were endeavoring to prove that the reducing power of plant juices as determined by titration with 2-6 dichlorophenolindophenol was due to vitamin C. Following quickly upon the determination of the chemical structure of vitamin C or ascorbic acid as it is now called, numerous papers have been published in which titration by this dye has been used for the quantitative determination of ascorbic acid.

Schlemmer, Bleyer and Cahnmann (6), Kieferle and Eisinreich (3), Birch, Harris and Ray (2), Bessey and King (1), and more recently many others have determined the amount of ascorbic acid in milk by titrating the filtrate with 2-6 dichlorophenolindophenol, after precipitating the proteins. Conditions requiring precipitation for the removal of interfering substances were discussed by Van Eekelen and Emmerie (11).

Milk apparently contains no appreciable amount of interfering substances and therefore acidified milk can be titrated directly without the removal of the proteins with perhaps more reliable results because adsorption and destruction of ascorbic acid during filtration are avoided. Duplicate determinations rarely deviate by as much as 1 mg. per liter of ascorbic acid and one person can make 40 determinations an hour. The recovery of added ascorbic acid is satisfactory provided the milk is not exposed to light except during the titration and is titrated immediately after the addition of the ascorbic acid. When 50.0 mg. of ascorbic acid per liter were added, the increase in ascorbic acid content of the milk from 5 different cows was found to be 49.7, 48.7, 50.7, 50.0, and 50.2 mg.

A detailed description of the method follows:

## 2-6 DICHLOROPHENOLINDOPHENOL SOLUTION

Place 0.3 gram first commercial preparations or 0.2 gram or less of later, more highly purified products of 2-6 dichlorophenolindophenol in a mortar. Grind to break up lumps and add 50 ml. of hot, distilled water. Grind further and decant through a filter into a one-liter volumetric flask. Add more hot water to the residue remaining in the mortar, and again decant onto the filter. Continue this process until the blue colored material has passed through the filter. Adjust to room temperature, and make up to the volume of one liter with distilled water.

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### SULFURIC ACID SOLUTION

A sulfuric acid solution of approximately 1/10 normal is used for acidifying and diluting the milk for titration. A stock solution of 10 normal is first prepared by diluting 285 ml. of concentrated sulfuric acid to one liter with distilled water. For use, 10 ml. of the stock solution is diluted with distilled water to make a volume of one liter. Approximately 25 ml. of this dilute solution is added to exactly 10 ml. of milk.

### STANDARDIZATION OF THE DYE SOLUTION

Weigh accurately, approximately 50 mg. of ascorbic acid. Place in a 500 ml. volumetric flask and make up to volume with distilled water. This solution should be used at once for standardizing the dye solution.

Introduce 5 ml. of the standard ascorbic acid solution into a 200 ml. beaker. Add approximately 15 ml. of the dilute sulfuric acid solution and 15 ml. of distilled water, and at once titrate with the dye solution to a light pink color, permanent for at least 30 seconds. Subtract from this titration value a blank determination made on the acid-water mixture brought to the same color. A blank of approximately 0.3 ml. is usually obtained. The amount of ascorbic acid in the 5 ml. aliquot, divided by the number of ml. of dye solution required, gives the mg. of ascorbic acid corresponding to 1 ml. of the dye solution. This value times 100 gives, in terms of mg. per liter, the amount of ascorbic acid corresponding to each ml. of dye solution required for the titration, when a 10 ml. sample of milk is taken for titration. The original amount of dye taken should be so adjusted or the solution so diluted that this factor falls in the limits of 5 to 8.

### TITRATION OF THE MILK

Pipette 10 ml. of milk into a 200 ml. beaker. Add 25 ml. of the dilute sulfuric acid solution and at once titrate with the dye solution, using a 10 ml. burette calibrated in 0.05 ml. divisions. In titrating the milk it will be found that after a small amount of the dye is added the milk will assume a pink color. Upon standing for a few seconds, this pink color will fade. More dye can then be added. This procedure is continued until a definite pink color remains for at least 30 seconds. This is taken as the end-point of the titration. After this end-point has been reached, the pink color will remain for several minutes. A blank, which is usually about 0.4 ml., should be subtracted. This blank is determined by allowing milk to stand until the ascorbic acid disappears when a constant low value is reached. This blank may be determined by allowing cold milk to stand for several days before it is titrated. The disappearance of the ascorbic acid in the milk can be accelerated by adding enough copper sulphate to make about a mg. per liter of Cu, or by placing the milk in sunlight.

Since in the early stages the titration is slow because of the fading end-point, it is very convenient to have six burettes and titrate six samples at once. In this way the dye can be added to the samples in rotation, while waiting for fading of the color.

Calculate results to the basis of mg. of ascorbic acid per liter of milk.

#### PRECAUTIONS

The dye solution is not stable, and on the average will decrease about 1 per cent in strength per day. It should therefore be re-standardized at 2 to 4 day intervals, and its strength interpolated to daily values.

Dye solutions over two weeks old do not give sharp end-points and therefore should not be used.

The ascorbic acid solution is not stable under ordinary conditions. It should be used within one to five minutes after preparation.

The addition of acid to the milk accelerates the destruction of the ascorbic acid. The milk should therefore be titrated as soon as the acid is added.

The exposure of the milk to light should be reduced to the minimum required for measuring and titrating the samples. See papers by Mattick and Kon (5) and Kon and Watson (4).

#### EXPERIMENTAL RESULTS

This method was used in obtaining the results previously reported by Sharp (7) and by Sharp, Trout and Guthrie (8).

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# FURTHER INVESTIGATIONS IN CHOPPING ALFALFA HAY AT THE TIME OF STORAGE

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## EFFECT ON SPACE REQUIRED, TEMPERATURES ATTAINED, COLOR, FEEDING VALUE, AND LOSSES OF FEED CONSTITUENTS

In an experiment (1) conducted by the Bureau of Dairy Industry at the National Agricultural Research Center, Beltsville, Md., in 1935, two lots of field-cured alfalfa hay, one containing 25.31 per cent moisture and the other containing 27.02 to 27.42 per cent moisture, were each stored in an open mow, both in the chopped form and in the natural long form.

Excessive heating and loss of green color and carotene occurred both in the long hay and in the chopped hay. The chopped hay heated more and lost more of its green color and carotene than the long hay of the same lot. Coarsely chopped hay ( $\frac{3}{4}$ -inch lengths) did not reach as high a temperature or remain hot as long as finely chopped hay ( $\frac{1}{4}$ -inch lengths). Coarsely chopped hay was light brown in color and finely chopped hay was darker brown to nearly black in color. The black chopped hay was not so palatable to dairy cows as the greener long hay, but the brown chopped hay was fully as palatable. However, the chopped hay was lower in feeding value for milk production than the long hay.

This experiment indicated that 25 to 27 per cent moisture is too high for either chopped or long alfalfa hay stored in an open mow; that chopped alfalfa hay stored in an open mow should be lower in moisture content than is necessary for long hay; and that hay should be coarsely chopped rather than finely chopped.

The present study was conducted in 1936, with alfalfa hay containing a lower moisture content (15.80 per cent). Only one lot of hay was used, half of which was put in the hay barn in the long form and half in a coarsely chopped form. The object was to determine the effect of a low moisture content (under the conditions of storage used) on the preservation of color, carotene, and feeding nutrients in coarsely chopped and long hays. The chopped hay was stored in a compartment having a floor space of  $8 \times 12$  feet. The long hay was stored in a compartment having a floor space of  $12 \times 20$  feet.

First-cutting alfalfa hay was mowed June 1, raked with a side delivery rake June 2, turned with a side delivery rake the morning of June 3, and put in the mow June 3 beginning at 1 P.M. The weather while the hay was curing was warm and clear with a good breeze. It rained in the middle of the afternoon while the hay was being hauled, limiting the quantity of hay

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that could be stored under comparable conditions to six loads. This was less hay than had originally been planned for storage. Alternate loads were stored long and chopped, making 3 loads, or 3 tons of each kind of hay. The chopped hay was cut in  $\frac{3}{4}$ -inch lengths, and blown into the compartment. At the time of storage, the hay was  $7\frac{1}{2}$  feet deep in each compartment.

Samples of the hays were taken for chemical analyses, carotene analyses, and color determinations. In each compartment a  $\frac{1}{2}$ -inch galvanized pipe was inserted at the center so that the lower end was about level with the top of the first load of hay put in. Ordinary mercury thermometers were lowered into these pipes and left there continuously except when removed for the daily temperature readings.

The hay was green, leafy, and well cured when placed in storage, and it contained only 15.80 per cent moisture. This is drier than is often the case when hay is put in the mow or stack.

#### METHOD OF FEEDING

Feeding was started after the hay had been in storage 150 days. Chopped and long hay were fed concurrently by the single reversal system to two groups of three Holstein cows each. The cows were fed all they would eat of each kind of hay for a 28-day period. Sufficient grain was fed in addition to provide a slight excess of total nutrients over the quantity called for by the Haecker feeding standard.

#### RESULTS

##### *The Mow Space Occupied*

The amount of space occupied by each hay when it was put in storage and when it was taken out is shown in Table 1. Both the chopped and the

TABLE 1

*Mow space occupied by chopped hay and long hay when put in storage and when taken out*

	QUANTITY OF HAY	STORAGE SPACE PER TON	DRY MATTER		
			Content	Per ton	Per cubic foot
	<i>Tons.</i>	<i>Cubic feet</i>	<i>Per cent</i>	<i>Pounds</i>	<i>Pounds</i>
Chopped hay:					
Hay put in storage .....	3.17	227	84.20	1,684.0	7.42
Hay removed from storage	2.88	222	88.14	1,762.8	7.94
Long hay:					
Hay put in storage .....	3.04	592	84.20	1,684.0	2.84
Hay removed from storage	2.72	529	88.94	1,778.8	3.36

long hay lost a little weight, due partly to a loss of moisture and partly to a loss of dry matter. Because of settling and the higher dry-matter content, both hays contained more dry matter per cubic foot when they were taken out of storage than when they were put in.

At the time of storage a ton of the chopped hay occupied 38 per cent as much space as a ton of the long hay. When taken out a ton of the chopped hay occupied 42 per cent as much space as a ton of the long hay.

### *The Maximum Temperature Attained*

The chopped hay reached a maximum temperature of 106° in 2 days, and in 9 days the temperature had dropped to 86° and in 16 days to 76° F. The long hay reached a maximum of 81° in 24 hours and dropped to 72° in 48 hours.

### *Influence of the Method of Storage on the Carotene Content*

When put in storage, each hay had a carotene content of 76 parts per million on a dry-matter basis. When the hay was removed, the carotene content of the chopped hay was 20.7 parts per million and that of the long hay 31.9 parts per million. In both hays the carotene content was apparently quite uniform from the top to the bottom of the pile.

The storage period included all the summer months. Investigations by the California (2) and Arizona (3) State Agricultural Experiment Stations and by the Bureau of Dairy Industry (4, 5) have shown that over equal periods of time, alfalfa hay loses more carotene during storage at high temperatures than at low temperatures. No doubt if this hay had been stored in the fall instead of in the spring, it would have retained more of its carotene.

### *The Effect on the Color<sup>1</sup>*

At the time of storage both hays were of an excellent green color. No determinations for percentage of green color were made at that time. After 5 months in storage, the long hay had 54 to 57 per cent as much green color as the greenest commercially-produced alfalfa hay, and the chopped hay had 47 to 48 per cent as much. The percentage of green color in both hays was uniform from the top to the bottom of the pile. Apparently the green color was destroyed at a slower rate than the carotene.

### *Influence on the Losses of Weight and of Dry Matter*

The weights of the two lots of hays and of the dry matter, at the time of storage, the weights at the time of removal from storage, and the losses in weight and dry matter during storage are shown in Table 2. The chopped hay lost 4.86 per cent of its dry matter and the long hay lost 5.33 per cent. Chemical analyses of the hays made at the time of storage and after 5 months of storage, applied to the weights given in Tables 1 and 2, show that dry-matter losses in both the hays consisted principally of nitrogen-free extract, with small losses of crude protein and ash and no losses of crude fiber or fat.

<sup>1</sup> Color determinations were made by Carl F. Welsh, Bureau of Agricultural Economics.

TABLE 2

*Weights of hay and dry matter put in storage, the weights taken out, and the losses in weights and dry matter*

	CHOPPED HAY	LONG HAY
Weight of hay stored . . . . . pounds	6,335.00	6,080.00
Weight of hay removed . . . . . pounds	5,758.00	5,449.00
Loss in weight during storage . . . . . pounds	577.00	631.00
Loss in weight during storage . . . . . per cent	9.11	10.38
Moisture content of hay stored . . . . . per cent	15.80	15.80
Moisture content of hay removed . . . . . per cent	11.86	11.06
Dry matter in hay stored . . . . . pounds	5,334.07	5,119.36
Dry matter in hay removed . . . . . pounds	5,075.10	4,846.34
Loss of dry matter . . . . . pounds	258.97	273.02
Loss of dry matter . . . . . per cent	4.86	5.33

### *The Effect on the Feeding Value*

The cows used in this experiment had been on pasture until October 22. They were then fed a ration consisting of corn silage, soybean hay, and grain until the hay feeding experiment began November 3. Because the quantity of hay available for the feeding trial was small, the cows were limited to an 8-day preliminary period and a 20-day experimental period on each kind of hay. The quantities of hay and grain consumed, milk and butterfat produced, and body weights are shown in Table 3. While such an experiment will show the relative palatability of the two hays and will give some indication of their value for the production of milk, it will not, of course, provide conclusive evidence of their relative value for extended feeding periods.

*Palatability of Hay and Quantities Eaten.*—The cows in this experiment did not eat as much hay as the cows in the 1935 experiment. Each cow was offered an excess of hay every day over what she would consume. The refused hay was weighed back once daily, and it averaged 15.0 per cent of the chopped hay offered and 14.5 per cent of the long hay.

Group A consumed 26.8 pounds of chopped hay per cow daily during the first period and 25.7 pounds of long hay during the second period. Group B consumed 28.5 pounds of long hay per cow daily during the first period and 25.2 pounds of chopped hay during the second period. For both groups, consumption of long hay averaged 27.1 pounds per cow daily, while consumption of chopped hay averaged 26.0 pounds. The excess of hay offered may have been responsible for the greater consumption of the long hay than of the chopped hay, because the long hay afforded better opportunity for the cows to select the choice portions. For this reason, both hays can perhaps be considered equal in palatability.

*Production.*—The difference in production on the two kinds of hay was very slight—probably no greater than would be expected from similar groups similarly fed. For this reason the difference is not significant and does not indicate a superiority of one hay over the other.

TABLE 3  
Summary of feed eaten, production of milk and butterfat, and body weights

	TOTAL FEEDS EATEN BY GROUP			TOTAL PRODUCTION BY GROUP		INCREASE (+) OR DECREASE (-) IN MILK PRODUCTION	BODY WEIGHT	
	Concen- trates	Hay	Total di- gestible nutrients	Milk	Butterfat		Average per cow	Average gain per cow
	Pounds	Pounds	Pounds	Pounds	Pounds	Per cent	Pounds	Pounds
Chopped hay:								
First 20-day period (Group A) .....	600.0	1,607.9	1,262.4	2,007.2	70.13	+4.6	1,236	14
Second 20-day period (Group B) .....	500.0	1,513.2	1,140.2	1,571.0	59.21	-4.4	1,144*	12
Total .....	1,100.0	3,121.1	2,402.6	3,578.2	129.34	+0.1†		13
Long hay:								
First 20-day period (Group B) .....	520.0	1,707.7	1,262.2	1,669.1	59.76	+0.1	1,150	11
Second 20-day period (Group A) .....	559.0	1,544.7	1,207.3	1,847.8	67.04	-0.1	1,251	12
Total .....	1,079.0	3,252.4	2,469.5	3,516.9	126.80	0.0†		12

\* Average for 2 cows. One cow's weight was not normal because of hip injury.

† Average for 20-day period.

Group A produced 33.5 pounds of milk and 1.17 pounds of butterfat per cow daily on the chopped hay during the first period, and 30.8 pounds of milk and 1.12 pounds of butterfat on the long hay during the second period. Group B produced 27.8 pounds of milk and 1.00 pound of butterfat per cow daily on the long hay during the first period, and 26.2 pounds of milk and .99 pound of butterfat on the chopped hay during the second period.

For both groups, production averaged 29.9 pounds of milk and 1.08 pounds of butterfat per cow daily on the chopped hay, and 29.3 pounds of milk and 1.06 pounds of butterfat on the long hay. Apparently the two hays were equal in value for milk production. The quantity of grain fed averaged 9.2 pounds per cow daily on the chopped hay, or 1 pound for each 3.25 pounds of milk; and 9.1 pounds on the long hay, or 1 pound for each 3.22 pounds of milk.

*Decline in Production.*—The rate of decline in production during the 20-day experimental periods, as shown in Table 3, is practically the same for the two hays. The percentage of decline was calculated from the first 3 and the last 3 days of each 20-day period. Group A cows increased 4.6 per cent in milk production on the chopped hay during the first period, with only a 0.1 per cent decline on the long hay during the second period. Group B cows increased 0.1 per cent in milk production on the long hay during the first period, with a decline of 4.4 per cent on the chopped hay during the second period. The slight difference in the declines is not sufficient to indicate the superiority of one hay over the other.

*Body Weights.*—Group A cows weighed more on an average than Group B cows. One Group B cow received a hip injury during the period she was on chopped hay. This injury affected her weight considerably, but had little or no effect on her feed consumption or milk production. The weights for Group B cows on chopped hay are therefore based on an average of two animals instead of three. The normal weight of the injured cow was within 5 pounds of the average of these two animals.

Group A cows gained 0.7 pound per head daily during period 1 (on chopped hay) and 0.6 pound during period 2 (on long hay). Group B cows gained 0.55 pound per head daily during period 1 (on long hay) and 0.6 pound during period 2 (on chopped hay). For both groups, the average gain per head daily amounted to 0.65 pound on chopped hay and 0.60 pound on long hay.

#### DISCUSSION

The present investigation, together with the 1935 experiment (1), emphasizes the point that high temperatures developed in storage are more destructive to the carotene content in both high and low moisture alfalfa hays than low temperatures. The work of the California station (2) also shows temperature to be a major factor in loss of carotene in alfalfa hay or meal during storage. Excess moisture in hay brings about conditions favorable for oxidation and excess heating in hay. To preserve the color and carotene content

of stored hay it is necessary to reduce the moisture content of the hay, before storing, to a point where excess heating will not take place under the storage conditions used.

In the 1935 experiment, long first-cutting hay with a moisture content of 25 per cent, stored in a mow to a depth of over 8 feet, reached a maximum temperature of 120° F., remained above air temperature for a 3-week period, and lost 93 per cent of the carotene it contained. Coarsely chopped hay of the same moisture content which was similarly stored, reached a maximum temperature of 128° in 19 days, did not return to air temperature for 2 months, and lost 95 per cent of its carotene.

In the present experiment, coarsely chopped hay with 15.8 per cent moisture that was stored in a mow to a depth of 7½ feet, lost considerably more carotene than long hay from the same lot that was stored under the same conditions. The chopped hay, reaching a maximum temperature of 106° which declined to 76° in 16 days, lost 72.8 per cent of its carotene during the 150-day storage period; whereas, the long hay, reaching a maximum temperature of only 81° which declined to 72° in 2 days, lost only 58.0 per cent of its carotene in 150 days of storage. It is apparent that there is a direct relationship between the temperatures attained in storage and the losses of carotene.

#### SUMMARY AND CONCLUSIONS

On June 3, 1936, first-cutting alfalfa hay containing 15.8 per cent moisture, nicely cured without rain or excessive loss of leaves, was stored in an open mow to a depth of 7½ feet in both the coarsely chopped and natural long forms. The hay was left in storage for 150 days.

Two and a half times as much hay was put in a given space in the chopped form as in the natural long form.

The long hay attained a maximum temperature of 81° F. the day after it was stored and declined to 72° in 2 days. The chopped hay reached a maximum temperature of 106° in 2 days and declined to 76° in 16 days.

The carotene content of both the long and the chopped hay was 76 parts per million of dry matter at the time of storage. After 150 days of storage the carotene content of the long hay was 31.9 parts per million and that of the chopped hay 20.7.

After 5 months of storage, the long hay had 54 to 57 per cent as much green color as the greenest grade of alfalfa hay and the chopped hay had 47 to 48 per cent as much.

Both the long hay and the chopped hay in this experiment lost green color at a slower rate than they lost carotene.

In the investigation of the previous year with chopped hay containing 25 per cent moisture, the green color was completely destroyed and the final carotene was only one-fifth as much as in the chopped hay in this investigation.

Dry matter losses were moderate and about equal in both kinds of hay. The chopped hay was as palatable as the long hay.

The quantities of milk produced and the maintenance of milk flow were very slightly in favor of the cows fed the chopped hay, in spite of their slightly lower consumption of nutrients as estimated from actual analyses. However, these slight differences in production might be expected with hay that is practically identical in quality.

This investigation indicates that hay with 16 per cent moisture will lose more of its color and carotene if stored in a chopped form than if stored in a long form.

The results of the 2 years' work show that the chopping of field-cured alfalfa at the time of storage in a mow is practicable if precautions are taken to see that the hay is not too high in moisture content. The principal advantages of chopping are: (A) More hay can be stored in a given space; (B) less work is required in the mow at the time of storage; (C) the hay can be removed from the mow more easily; and (D) in the case of stemmy hays, consumption is more complete because the leaves are not so readily selected from the chopped hay as from long hay.

To insure success with chopped hay certain precautions must be observed. In the first place the hay to be chopped should be dried in the field to a low moisture content. While this is important when the hay is to be stored in the usual way, it is doubly important when the hay is to be chopped. Furthermore, the hay should be chopped into  $\frac{3}{4}$ -inch lengths or longer. If these precautions are observed, the quality of hay made by storing it in a chopped form will be approximately equal to the quality of hay made by storing it in the natural long form, except that even under the best practicable conditions the chopped hay will still lose more of its color and carotene than the long hay.

Both high-moisture content and fine chopping increase the temperature of stored hay and lead to excessive destruction of color and carotene and to a lowering of the feeding value. Under certain conditions the temperature may become high enough to make the chopped hay a fire hazard.

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## American Dairy Science Association Announcements

THIRTY-THIRD ANNUAL MEETING, OHIO STATE UNIVERSITY,  
COLUMBUS, OHIO AND OHIO AGRICULTURAL EXPERI-  
MENT STATION, WOOSTER, OHIO, JUNE 14-17, 1938

### *Monday—June 13*

1 P. M.—9 P. M.—General Registration.

### *Tuesday—June 14*

8 A. M.—10 A. M.—General Registration and Room Registration.

10 A. M.—12 NOON—Opening Session—H. W. Gregory, Presiding.

1 P. M.—2:30 P. M.—General Session—(Instruction).

2:30 P. M.—4:30 P. M.—Sectional Meetings:

Manufacturing Section, Dairy Products Judging  
Conference for Coaches and Instructors.

Production and Extension Sections. Symposium  
on Nutrition.

4:30 P. M.—Meeting of the Board of Directors, and Committee  
Meetings.

8:30 P. M.—Social Hour.

### *Wednesday—June 15*

8 A. M.—10 A. M.—General Registration and Room Registration.

8 A. M.—9:30 A. M.—Sectional Committee Meetings.

8 A. M.—9:30 A. M.—Inspection of Extension Exhibits.

9:30 A. M.—12 NOON—Sectional Meetings.

12 NOON—1 P. M.—Dairy Luncheon.

1 P. M.—4:30 P. M.—Sectional Meetings.

8 P. M.—Entertainment.

### *Thursday—June 16*

8 A. M.—9 A. M.—Committee Meetings.

9 A. M.—11:30 A. M.—Sectional Meetings.

11:30 A. M.—12 NOON—Sectional Business Meetings.

1 P. M.—3:30 P. M.—Sectional Meetings.

3:30 P. M.—4:30 P. M.—General Business Meeting.

6:30 P. M.—Annual Banquet.

### *Friday—June 17*

Wooster, Ohio

10:30 A. M.—12 NOON—General Session—Earl Weaver, Presiding.

12 NOON—1 P. M.—Luncheon.

1 P. M.—Points of Interest at the Ohio Agricultural Experi-  
ment Station.

Members of the Association are invited to submit titles and abstracts of  
papers dealing with original investigations. Those interested in presenting

papers should refer to page 789 of the December, 1937 number of the JOURNAL for pertinent information. Time allotted for each paper limited to 10 minutes. Titles and abstract of papers must be in the hands of the chairman of the program committee by April 15.

All communications relative to the program should be addressed to Dr. T. S. Sutton, Department of Animal Husbandry, Ohio State University, Columbus, Ohio.

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NOMINATIONS FOR THE 1938 BORDEN AWARDS FOR CONTRIBUTIONS  
IN THE DAIRY PRODUCTION AND DAIRY  
MANUFACTURING FIELDS

The recipients of the two annual Borden Awards, each consisting of \$1000 and a gold medal, for the most meritorious research in the field of dairy cattle production and the field of dairy manufacturing, will be selected by the awards committee of the American Dairy Science Association. Please send your nominations for these honors to the Secretary of the American Dairy Science Association, Dept. of Dairy Technology, Ohio State University, Columbus, Ohio, so that they will reach him not later than March 1, 1938. The awards will be made for (1) the most outstanding research work in the field of dairy production, breeding, feeding, or farm sanitation and other

phases of production work, and (2) for outstanding research in the field of dairy manufacturing, such as improvement in equipment or methods in the handling of milk or its products and the production of milk products. Nominations should be made for those whose work has been completed and published during the five year period ending December 31, 1937.

Those who are eligible for consideration for the awards must be under forty years of age at the time of the award.

Since Borden awards are also being made for meritorious work in basic science as related to, or as it affects the Dairy Industry; for work in basic research on vitamins or other nutritional aspects of milk products; for work in the practical application of the findings in nutritional research; and for work in public health as related to the Milk Industry, these awards to be administered by other scientific societies, these phases of research work as applied to the dairy industry should not be considered in making your nominations for research in the production and the processing fields.

Each member of the American Dairy Science Association is entitled to make nominations for these awards. Please give the following information in your nominations:

I, the undersigned member of the American Dairy Science Association  
 nominate .. . . . for the Borden award for  
 1938 in the field of dairy production, dairy manufacturing for his contribu-  
 tion entitled ..... and delivered before  
 or published in ..... Signed:

DAIRY PRODUCTION AWARDS COMM.      DAIRY MANUFACTURING AWARDS COMM.

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## THE PREPARATION, PROPERTIES, AND USE OF GONAD-STIMULATING HORMONES<sup>1</sup>

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The phenomenal results obtained from the use of gonad-stimulating hormones in the experimental laboratory have attracted widespread attention. It is a striking thing that a baby female rat can be made to ovulate 75 or 100 eggs when the best her mother ever did was to produce 10 or 12 eggs at one time. No other agent has been known which exerts such a clear and definite effect in speeding up the process of reproduction. People's minds have been challenged with the tremendous possibilities of modifying and controlling the process of reproduction in higher animals by means of these substances. Human medicine has been interested because of ever-present clinical problems which arise from aberrant sexual function. Animal husbandmen have seen in the discovery a possibility of dealing more effectively with the problems of sterility and lowered fertility in their livestock.

After the first report of the discovery, which was only about 10 years ago, work with the gonad-stimulating hormones has settled down chiefly to investigation of their sources, chemistry, and physiological action.

While the pituitary glands, body fluids, and certain other tissues of mammals, birds, and some lower animals all probably contain gonad-stimulating substances in some form and in some quantity, there are only a few sources from which sufficient quantity of the hormone can be obtained to justify their discussion here. The pituitary glands of hogs, sheep, cattle, and horses, which are slaughtered in large numbers at packing houses, constitute one important source. The urine of pregnant women and the blood serum of pregnant mares between the 40th and 80th days of gestation are also important sources. While there are other rich sources of hormones, such as foetal membranes and endometrium of pregnant mares, human placentae, urines of castrate men or women, menopausal women, and men or women with embryonal cell tumors, the sources themselves probably are not abundant enough to have widespread importance.

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The preparation of crude extracts from pituitary glands takes advantage of the solubilities of the hormones in water, dilute acids or dilute alkalis. After the active material is dissolved out of the tissue it can be removed from solution by precipitation with organic solvents such as alcohol or acetone, or by other substances such as tannic acid, tungstic acid, or ammonium sulfate. The active substance can also be adsorbed on such material as aluminum hydroxide. With repeated extractions and precipitations, meanwhile decreasing the volume of solvent, a fair degree of concentration and elimination of extraneous material can be brought about. Similar crude preparations from urine or blood serum rely first on precipitation and then repeated extractions and precipitations to bring about a partial purification. If certain precipitants are used, it may be necessary to employ special methods to free the active material from the precipitant, such as dialysis in the case of ammonium sulfate or treatment with brucine in the case of tungstic acid.

At least four different fractions of the pituitary extracts which have an effect on the gonads are at present recognized by some investigators. These have been characterized as follicle-stimulating, luteinizing, interstitial-cell-stimulating, and inhibiting in physiological action. The separation of the various fractions has been accomplished by fractional precipitation, usually by "salting-out," by increasing the hydrogen-ion concentration or by a combination of the two methods. No separation of extracts of urine from pregnant women into fractions showing different physiological action has been accomplished. Some evidence has been reported that pregnant mare serum extracts can be separated into follicle-stimulating and luteinizing fractions by methods similar to those used in fractionating the pituitary extract.

Physiological characterization of the various gonadotropic extracts has been made for the most part on the albino rat. In very general terms, the following reactions are obtainable in the ovary: (1) development of vesicular follicles to mature size or even larger, with accompanying female-hormone secretion; (2) ovulation of fertile eggs; (3) formation of functional corpora lutea; (4) luteinization of follicles without prior ovulation, thus forming corpora lutea atretica; and (5) development of interstitial tissue. The foregoing generalization must be qualified inasmuch as all of the responses mentioned are not invariably obtainable. For example, urine of pregnant women is relatively ineffective in hypophysectomized animals. Extracts from any source produce little effect in young animals before vesicular follicles form in the ovaries. Follicular stimulation can be brought about in younger animals than can ovulation or luteinization. Crude pituitary extracts tend to form atretic corpora lutea rather than corpora of ovulation. Graded dosages of pregnant mare serum have yielded quite different results: smallest dosages—follicular-stimulation; larger dosages—ovulation of a number of follicles normal for the species; still larger—superovulation; largest—formation of cystic follicles and atretic corpora lutea.

Mammalian testes have not responded to gonad-stimulating extracts to the same degree as mammalian ovaries. The most commonly recognized testicular response is that of increase in interstitial tissue and the accompanying hypersecretion of male hormone. The secretion of the testicular hormone has been assumed, more from the response of the accessory glands, such as the seminal vesicles, than from a marked increase of sexual desire. Consistent effects on the tubular tissue of the testes have not been reported. Some investigators have observed an increase in size of the seminiferous tubules and an increase in number of spermatocytes. Others, on the contrary, have observed actual tubular damage.

The situation with regard to the differential responsiveness of ovary and testis as observed in mammals appears to be reversed in birds. There the testes are quite sensitive to stimulation, even to complete spermatogenesis; whereas the ovaries are quite refractory. In some seasonal breeding mammals, however, such as the ground squirrel, the testes can be stimulated quite completely by gonad-stimulating extracts.

The various fractions of gonadotropic extracts are characterized, physiologically, by a preponderant action with relative, but not necessarily complete, absence of other actions. For example, a follicle-stimulating extract causes the maturation of follicles without formation of corpora lutea in only 2/3 to 3/4 of normal 21-day-old female rats. It has been postulated that the animals' own pituitaries have furnished the luteinizing action in the animals which form corpora lutea. Some evidence in support of this comes from the greater consistency of follicle-stimulation in hypophysectomized rats. The follicle-stimulating fraction when administered to males is said to be gametogenic, causing growth of the testicular tubules and development of their epithelium. The action appears to be more pronounced in restoring function in hypophysectomized mature rats than in hastening function in immature normal animals.

The action of the luteinizing fraction in the female becomes effective either immediately following the action of a follicle-stimulating preparation or in synergism with its action. Presumably, small vesicular follicles are not acted upon by the luteinizer. It is only as a follicular-stimulator develops the follicle nearly to mature size that the luteinizer, alone, or in interaction with the follicle-stimulating fraction, brings about the pre-ovulatory swelling of the follicle, ovulation, and corpus luteum formation.

Some investigators have considered the development of interstitial tissue in the testicle and the resulting secretion of male hormone as an action of the luteinizing fraction. Still other investigators believe this response can be attributed to a specific fraction, the interstitial-cell-stimulator, which produces relatively little luteinizing or follicle-stimulating action. In the female this fraction is effective in preventing the regression of the interstitial tissue of the ovary in hypophysectomized rats.

Another fraction of a pituitary extract is known as the gonadotropic antagonist. It can hardly be called gonad-stimulating in action inasmuch as

it inhibits the action of the follicular-stimulator. To be most effective it must be administered intraperitoneally rather than subcutaneously. It appears to act by causing atresia of follicles that might otherwise have been developed by the follicle-stimulating preparation, but has no inhibitory action upon luteinization.

The relatively specific actions of different gonad-affecting fractions have led to the postulation that the anterior pituitary gland secretes a number of different hormones affecting the gonads. Some of the fractions are spoken of as "hormones," without qualification. Hypotheses are made regarding the regulatory mechanism of the oestrous cycle. These are based on the action of certain pituitary hormones at certain times in the cycle, followed by the action of other hormones to take care of the next end-reaction observed in the ovaries. It seems only reasonable to point out in this connection that much of the experimental work which forms the foundation for these hypotheses has involved fractions of extracts and not "pure" hormones. Such preparations probably contain one or more hormones in unknown concentrations or proportions and in addition, undetermined amounts of substances which by themselves are relatively inert physiologically. The physiological action of the fraction as a whole, however, may be a result of a marked interaction of these inert substances with the primary substances in the preparation. In other words, a certain response from a pharmacological preparation does not necessarily imply a correspondingly identical substance acting in the physiological mechanism of a normal individual.

Two other types of substances, which are probably extra-pituitary in origin, should be considered at this time because they add complexity to the response of an experimental animal to hormone administration. The first of these may be classed as augmenters, inasmuch as they appear to increase the gonad-stimulating action when administered to immature rats of either sex in conjunction with some pituitary extracts. Augmenters include a variety of chemical substances such as hemin, casein, zinc, and copper salts, extracts of yeast, liver, muscle, lemons, etc. No entirely satisfactory explanation has been offered for the action of all these substances. Perhaps one of the most prevalent is that they prolong and slow up the rate of absorption from the site of injection or slow up the rate of removal of the hormone from the blood stream. The action of the augmenters in species other than the rat has not been adequately investigated.

The other type of modifying substance is the inhibitor. The antagonist of pituitary origin has already been mentioned. In addition to this, however, certain species have been found to build up anti-hormones in their blood serum when they are submitted to long-continued injections of the gonad-stimulating extracts. The anti-hormone exhibits itself by failure of the animal to respond further to the action of the hormone. It can also be demonstrated by the inhibiting or neutralizing effect when serum from a treated animal is mixed with the gonadotropic extract which induced the

anti-hormone and injected into test rats. The question arises as to the specificity of the anti-hormone. Some results seem to indicate that anti-hormones can be developed which will inhibit gonadotropic extracts from whatever source. Other results indicate that they are largely specific. Differences in the method of building them up and in the sensitivity of the test for them may account for the discrepancies.

While augmenters and inhibitors in the form in which they are being studied may not play any great part in the normal physiology of an animal, it is possible that they may have value for clinical use. One might conceivably use augmenters with the gonad-stimulating action of the pituitary gland in cases of hypo-gonadism and inhibitors in cases of hyper-gonadism.

The interest of this audience is doubtless centered on the possible use that gonad-stimulating hormones may have in correcting breeding troubles in dairy cattle. Those breeding difficulties in which infection is not the primary cause and in which abnormal gonadal function impairs fertility would appear to offer some possibility of responding to treatment with gonad stimulating hormones. Superficially, animals of this type might be placed in five classes: (1) cows that do not come into heat; (2) cows that come into heat more or less regularly but do not settle upon service; (3) nymphomaniac cows; (4) bulls that serve cows more or less readily but do not settle them; (5) bulls that will not serve a cow or are very slow in doing so.

A certain amount of breeding difficulty is encountered in any breeding herd. Attempts have been made to lessen these difficulties by injecting a part or all of the animals with gonad-stimulating extracts. In some of these cases the number or seriousness of breeding difficulties has actually increased. In other attempts only those animals which were showing some indication of breeding difficulty were injected. The results have been inconclusive. Some cows conceived; other appeared to be made worse; still others were unaffected as far as could be told. It has been found that gonad-stimulating extracts are effective in cows in bringing about practically all of the ovarian changes which would appear to be necessary, such as maturation of follicles, ovulation, formation of corpora lutea. The problem is to control these reactions so as to overcome the particular breeding difficulty involved.

Experimental work with rats has indicated that the qualitative and quantitative responses may be quite different with different dosages of gonad-stimulating extracts and different methods of administration. It is therefore necessary when applying these preparations to cows, that different dosages and different methods of treatment be studied. The cow presents a peculiar problem from the standpoint of hormone therapy because the ovulation of more than one egg at a time is highly objectionable. Gonad-stimulating extracts tend to develop a number of follicles, the degree of stimulation being related to the size of the dosage and the interval of treatment. It has been extremely difficult to bring a single follicle to maturity without other follicles being brought to a nearly comparable stage.

The cow herself is a second factor which renders control of hormone therapy difficult. It is not at all unlikely that every kind and degree of breeding difficulty will require a different treatment. Cows that may appear to have the same difficulty from the standpoint of behavior may represent widely diverse physiological conditions. As an illustration, it has been found at the Wisconsin Experiment Station that cows which fail to come into heat within a reasonable time after calving may have any one of the following pathologic ovarian conditions: (1) persistent corpus luteum; (2) quiescent ovaries, with neither follicles nor corpora lutea of appreciable size; (3) periodic development of anovulatory follicles, without heat; (4) periodic ovulation without heat. Even this type of analysis, with a knowledge of the ovarian response to hormones in the different conditions, may not be sufficient to give the control necessary for overcoming breeding difficulties. Each individual animal will doubtless need clinical study over a period of time to determine the indicated treatment and then the course of the treatment may have to be gaged to the response of the individual animal.

Investigation of this general problem at the University of Wisconsin during the past three years has been centered largely on the gonadal responses to hormonal treatment rather than on correcting breeding difficulties of herds in production. It was recognized that the animal's own endocrine system, and in particular the anterior pituitary, may complicate the study because of the additive or interactive effects of its own hormones. For that reason a part of the studies have been carried on with young calves, in which gonadotropic activity is probably at lower ebb than in mature individuals. It should be pointed out, however, that gonadal activity is exceedingly variable in calves even long before puberty. Very few calves of any age have shown a complete absence of macroscopically visible vesicular follicles and these have given no ovarian response to gonad-stimulating extracts. Ovulation has been brought about in other calves in three different ways: (1) injection subcutaneously of unfractionated pituitary extract, daily, for 11 or 12 days; (2) daily injection, subcutaneously, of pregnant mare serum for 6 days; (3) injection of follicular-stimulating extract, subcutaneously, on each of 2 to 6 days followed by administration of luteinizing extract intravenously on the succeeding day. The number of eggs ovulated has varied from 1 to 5 and many follicles which did not ovulate were definitely stimulated in each case.

Large quantities of sheep pituitary extract and of pregnant mare serum have been injected into bull calves one to four months of age over periods as long as 7 weeks but in no case have mature sperm been demonstrated. The size of the testicles was increased but it has not been definitely determined whether this was due to increase in interstitial tissue or size of seminiferous tubules or both. The seminal vesicles of these calves were definitely stimulated, which points to increased activity of the interstitial tissue. In

neither the bull nor the heifer calves has there been definite evidence of development of sexual desire even with extreme development of the gonads.

It is much more difficult to evaluate the effects of a gonad-stimulating extract when injected into mature cows. If the reproductive organs of the animals are normal, their ovaries and pituitary glands are changing periodically. If the animals are abnormal, it is very difficult to know which part of the endocrine system is primarily responsible for the condition. It may be possible that two animals which exhibit similar external behavior, and even similar internal behavior of the genital organs, are suffering from different fundamental defects of the endocrine system and will give different responses to the same endocrine treatment.

A general statement can be made that cows in all the different reproductive conditions which have been tried have responded in some degree to gonadotropic extracts. These include cows in the follicular and in the luteal phases of the oestrous cycle, in different stages of pregnancy, in the immediate post-partum period, cows with cystic ovaries, quiescent ovaries, and ovaries containing retained corpora lutea. The quantitative response to a standard dosage probably varies widely in animals representing the various foregoing pathologic conditions.

An entirely satisfactory measure for a dosage of gonadotropic extract is lacking. As long as the hormone is not in a chemical form that can be measured physically, there appear to be only two possible ways of expressing quantity of hormone. One is in equivalence of tissue or body fluid from which the hormone came and the other is in units determined by degree of response in small laboratory animals. Both methods are subject to criticism but the latter method is generally preferred because it gives some expression of the potency of a preparation. It is objectionable, however, in that it measures activity in a species other than the one in which it is to be used. Equal rat unit dosages of unfractionated pituitary extract and of pregnant mare serum give quite different results in cattle, fewer rat units of pituitary extract being needed to produce a response. Even among pituitary extracts there is some indication that similarly prepared extracts, administered in equal dosage in terms of rat units, may give quite different quantitative responses in cattle. This general situation may be due in part to error of the biological assay in each of the species; it may also be that undetermined interacting substances may affect the response in one species and not in the other.

Superovulation has been brought about in cows by subcutaneous injections of unfractionated pituitary extract just before an expected heat period. As many as 19 follicles have been ovulated from such a treatment. Although the ovaries have been enlarged by the treatment in all cases, in some only one follicle has been ovulated and in others luteinization of follicles has occurred without any ovulation. The conclusion is that the control of ovulation by this treatment does not appear satisfactory.

It should be noted at this point that workers at the California Experiment Station report ovulation being produced in the cow within 72 hours after the administration of pregnant mare serum.

Probably the surest method of producing ovulation with unfractionated pituitary extracts is by subcutaneous injections for one to seven days to grow the follicles and then intravenous injection of the same preparation two days later to produce ovulation. Ovulation has occurred within 36 to 48 hrs. after the intravenous injection. To a certain extent the number of follicles matured and subsequently, the number of eggs ovulated appears to be controllable by the total dosage given subcutaneously. But even by reducing the dosage the ovulation of a single egg is not insured. A single intravenous injection of the pituitary extract has not produced ovulation even though there has been a mature-sized follicle in the ovary, developed by the animal's own hormonal action.

It is doubtful if a single heat period has been observed in the experimental animals which can be ascribed to the hormonal treatment. A complete set of ovarian changes can be brought about and yet the cow shows no inclination to accept the bull. In some cases when the treatment has been near the time of the cow's expected heat period, she has accepted the male satisfactorily. Such heat periods are sometimes extended in length or they may be intermittent for a few days. The situation seems to emphasize the need for study of the physiological factors which condition the oestrous behavior of the animal as well as of those that bring about the ovarian changes.

All these results make it clear that there is much we do not understand about the action and standardization of gonad-stimulating hormones, let alone their effective use and control. Therapeutic use of these substances will undoubtedly sometime have a place in dealing with cases of sterility and lowered fertility in dairy cattle. At the present time, however, we are to a large extent dealing with preparations of unknown purity and unknown strength. Quite variable results have been obtained from administration of these preparations to different cows. Preparations of consistent strength and purity are highly desirable. It also seems necessary to know more about the altered physiology of the reproductively abnormal animal and the responses of each specific kind of abnormality to different methods of treatment (medical or surgical). Although successes from use of these materials on such animals are largely a result of chance at the present time, systematic study of these cases so as to recognize clinical entities should eventually give greater control in obtaining the desired results. Another outcome which should result from investigation of this field is the discovery of causes of reproductive abnormalities and subsequently, means to prevent their development. Such an outcome is even more important than the perfection of hormonal therapy for breeding difficulties.

# A COMPARISON OF FRESH AND FROZEN CONDENSED SKIM-MILK AS A SOURCE OF SERUM SOLIDS IN ICE CREAM<sup>1</sup>

E. L. REICHART AND R. T. CORLEY<sup>2</sup>

## INTRODUCTION

In ice cream making it has been customary to use concentrated milk products such as plain condensed skimmilk, sweetened condensed skimmilk, and skimmilk powder in order to build up the serum solids content of the mix. This has been done in order to improve the texture and body of the ice cream, and because such concentrated milks are a relatively cheap source of solids.

As there are certain seasons of the year when milk supplies are plentiful while at others there is a shortage, it would be desirable to store milk solids with a minimum amount of processing prior to storage.

Milk solids-not-fat can be stored in various forms; the possibility of storing serum solids in the form of frozen condensed skimmilk has been tried and found quite successful, as a commercial practice, in the University Creamery.

## STATEMENT OF PROBLEM

The purpose of this study was to study the effect of using frozen condensed skimmilk of good quality in ice cream.

Since the length of time taken to reach a certain percentage or a maximum overrun during the freezing process, and the quality of the resulting ice cream are the two most important points in which the average manufacturer is interested, any differences in these points obtained as a result of the use of frozen condensed skimmilk in ice cream mixes, were especially noted. Any other observable variations in the ice cream mixes themselves were also carefully noted. Therefore this study was a tabulation of the changes occurring in ice cream mixes in pH, titratable acidity, protein stability, whipping ability, and quality from the standpoint of flavor, body and texture, and resistance to melting, that took place as a result of the use of frozen stored condensed skimmilk as a source of serum solids. No attempt was made to study the problem from a bacteriological point of view.

## REVIEW OF LITERATURE

Webb and Hall (13) found that fresh whole milk could be pasteurized, condensed to one-third its weight, canned, and frozen, without any detri-

<sup>1</sup> Published with the permission of the Director as technical article No. 203 of the Nebraska Agricultural Experiment Station.

<sup>2</sup> The data presented in this paper are from a study made by the junior author under the supervision of the senior author in partial fulfillment of the work required for the degree of Master of Science.

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mental effects to the body or flavor of the product. These investigators also found that skimmilk of 9 per cent solids was unchanged in heat stability (time required for heat coagulation at 120° C.) after storage for 33 weeks in a frozen condition whereas milk of 18 per cent solids did not possess any stability toward heat at the seventeenth week. Anderson and Pierce (1) showed that holding milk at -25.7° C. (-14° F.) gave no precipitated protein till the third month and was usable till the sixth month whereas milk held at -12.5° C. (10° F.) showed some protein precipitation at the end of the second month and at the end of the fifth month practically all protein had precipitated out. Doan and Baldwin (4), referring to the work of Webb and Hall, state that freezing, as such, has no measurable effect upon protein dispersion but rather that holding in a frozen condition, for several weeks or months, is required to definitely cause aggregation or instability of the proteins. Mack (5) found that the proteins in mixes made from frozen cream were less stable than those in mixes made from fresh cream, as measured by the protein stability test used by Doan (2). Ramsey (9) stated that the use of frozen condensed skimmilk in ice cream mixes to be used in continuous freezers, was unsatisfactory due to the denaturation of the proteins as a result of freezing. Ramsey, in a communication to the junior author (10), states that while the use of frozen condensed skimmilk in mixes to be used in batch freezing has proved fairly satisfactory, such mixes cannot be used in Vogt Freezers due to the poor quality of the ice cream produced. Doan (3) concluded that when frozen condensed whole milk is stored more than eight weeks, the proteins become denatured and a gelation occurs, when the milk is thawed.

#### EXPERIMENTAL METHODS

##### *Preparation and Study of the Condensed Skimmilk*

For the manufacture of the condensed skimmilk to be used in this study, skimmilk was taken immediately after separation from pasteurized whole milk and heated to a temperature of 170° F. for forewarming. It was then drawn into a Rogers 18-inch copper vacuum pan, where it was condensed to approximately one-fourth of the original weight, under a vacuum of about 23 inches. After testing for fat and solids, it was run into new five-gallon tinned lard cans and stored at approximately 0° F. The condensed skimmilk contained 36.76 per cent total solids and .32 per cent fat. A total of 9 cans was put into storage—one to be taken out each month, from September on through May.

As each monthly batch was withdrawn from storage and melted, by allowing to stand at room temperature for 48 hours or by setting in cold water, observations were made for possible changes in the physical structure of the condensed milk or presence of "off-flavors" that might have developed during the storage period.

The fresh condensed skimmilk used in each monthly batch was tested for solids and fat as soon as made. The mix containing this fresh condensed skimmilk was made up the same day and from identical ingredients in order to avoid variation in the composition.

### *Preparation of the Ice Cream Mixes*

Once each month, for nine months, two 400 lb. batches of mix were made. One mix contained fresh condensed skimmilk as the concentrated source of serum solids while the other contained the condensed skimmilk that had been frozen and stored. Each mix was pasteurized at 150° F. for a period of thirty minutes, homogenized with a two-stage, 60-gallon per hour Colony homogenizer at a total of 3500 pounds per square inch pressure, with 500 pounds pressure on the second stage, and run over a surface cooler and cooled to approximately 40° F. The mix was run into 10-gallon milk cans and held below 40° F. till used.

The mixes were calculated to contain 14% butterfat, 10% serum solids, 15% sugar, .25% gelatin, and 39.25% total solids.

The ingredients were weighed carefully with the purpose of keeping the mixes as nearly alike as possible. In addition to the condensed milk used, the only other ingredients going into the mixes were fresh cream, fresh skimmilk, sugar, and gelatin.

### *Analytical Procedure*

The butterfat and total solids content were determined by use of the Mojonnier method (7).

The pH of the ice cream mixes was determined by means of the Coleman pH electrometer, using a glass electrode (8), at 25° C. (77° F.). The titratable acidity determinations were made as outlined by Sommer (11).

Protein stability (2) of the samples was rated relatively, according to the least amount of alcohol (sp. gr. 0.8912 at 20° C.) required to produce the first noticeable trace of flocculation in 5 cc. of sample. In all cases water was added prior to the alcohol so that the total volume of the addition (water plus alcohol) amounted to 10 cc.

### *Method of Freezing*

The ice cream mixes were frozen in a Creamery Package 40-quart horizontal brine freezer. The two comparable batches of mix which had been held at about 40° F. until used, were frozen, the mix containing the fresh condensed skimmilk always being first. A preliminary batch was always frozen in order to have the freezer cold and to whip each mix as nearly the same as possible. At the start of freezing 20 cc. of vanilla isolate extract were added to each batch of mix. The brine was shut off when the temperature was lowered to 22.6° F. Overrun readings were taken at minute intervals after the brine was shut off.

### *Method of Procuring Samples*

Samples were obtained by filling quart Sealright containers—(two from each batch taken directly from the freezer) as soon as 100 per cent overrun was obtained, after which the remainder of the batch was run into parchment-lined five-gallon ice cream cans. The samples and cans were immediately transferred to the hardening room which was kept at a temperature of approximately 0° F.

### *Judging the Ice Cream and Determining Its Melting Quality*

The quart samples were removed from the hardening room about two weeks after freezing and were cut in half. One pint of each quart was scored<sup>a</sup> for flavor and body. The remaining pint of the original quart sample was set upon a  $\frac{1}{4}$ -inch mesh wire resting on the rim of a 5-inch heavy glass funnel with the end leading into a 100 cc. graduate cylinder. The number of minutes it took for 100 cc. of the melted ice cream to collect in the cylinder was recorded for each sample. The character of the liquid in the cylinder and the amount of foam in the cylinder were also observed. Each batch was, therefore, represented by duplicate samples and the average of the two duplicates represented the figure taken for each sample.

## EXPERIMENTAL RESULTS

### *Effect of Freezing and Storing upon the Milk Protein*

During the nine months of the experiment, no change in appearance of the frozen condensed milk was noticeable, when it was first removed from the storage room. However, when it had been melted down it was observed that at the third month the condensed milk was coarser than when first made, and at the fourth month a partial gel had been formed. From the fifth month on, the milk had formed a complete gel and in addition, at the eighth and ninth months, some "wheying-off" was observed on thawing. The gel formation was readily broken up and dispersed when put into the vat as part of the mix and no trouble was experienced with it throughout the processing of the mix.

Table 1 shows that the number of cubic centimeters of alcohol required to give the first noticeable flocculation in each mix was comparatively constant all of the way through the experiment with the stability of the mixes containing the frozen condensed skim milk consistently below that of the mixes containing the fresh condensed skim milk. The amount of alcohol required to coagulate the frozen condensed mixes was 6.5 cc. for each determination except for the fourth, eighth and ninth months, when it was respectively 7.0 cc., 6.0 cc. and 6.0 cc. The fresh condensed mix always required 7.0 cc. to give coagulation.

<sup>a</sup> All samples were scored by Dr. P. A. Downs of the Department of Dairy Husbandry, University of Nebraska.

TABLE 1

*Protein stability (to alcohol) of ice cream mixes containing fresh and frozen condensed skimmilk*

Fresh		Frozen	
Mix No.	Alcohol No.*	Storage period in month	Alcohol No.*
1 . . . . .	7.0	1	6.5
2 . . . . .	7.0	2	6.5
3 . . . . .	7.0	3	6.5
4 . . . . .	7.0	4	7.0
5 . . . . .	7.0	5	6.5
6 . . . . .	7.0	6	6.5
7 . . . . .	7.0	7	6.5
8 . . . . .	7.0	8	6.0
9 . . . . .	7.0	9	6.0

\* Alcohol number is the number of cc. alcohol required to give the first flocculation in 5 cc. of mix.

*Effect of the Use of Frozen Condensed Skimmilk on the  
Acidity of the Ice Cream Mixes*

In comparing the results of hydrogen-ion concentration determinations, no significant difference was observed between those of the mixes made using frozen condensed skimmilk stored for various intervals and those using fresh condensed skimmilk.

The titratable acidities varied slightly from batch to batch, and sometimes, from day to day as would be expected, but the differences were at no time large enough to be significant.

*Effect of the Use of Frozen Condensed Skimmilk upon the  
Whipping Ability of Ice Cream Mixes*

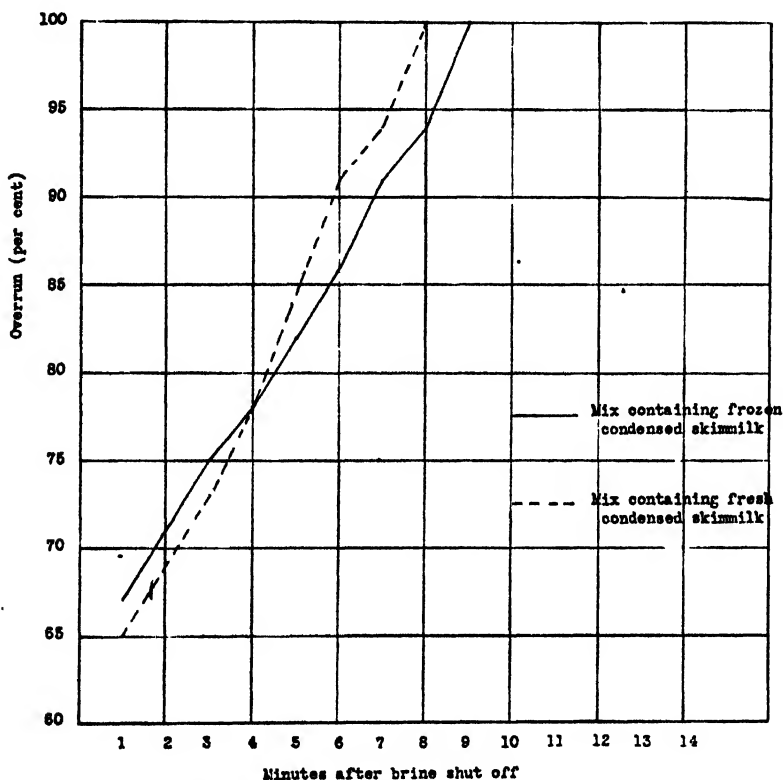
Table 2 shows overrun readings at minute intervals comparing the different batches of mix made from fresh and frozen condensed skimmilk over a nine months' period. These data are summarized in Graph 1 and show that at the end of one minute, the mix made from fresh condensed skimmilk was less in overrun, and this was continued in general, until between three and four minutes the overrun of both mixes became equal, being about 78 per cent. From that time on the conditions changed and the fresh condensed skimmilk mix showed the more rapid increase in overrun percentage, reaching its 100 per cent in eight minutes after the brine was shut off, whereas the mix containing frozen condensed skimmilk did not reach 100 per cent overrun until nine minutes after the brine was shut off. This difference was not large enough to warrant the condemnation of the use of frozen condensed skimmilk; it merely suggested that 100 per cent overrun is reached a little slower with frozen condensed milk than with fresh.

TABLE 2  
*Overrun freezing record of ice cream mix containing fresh and frozen condensed skim milk*

Mix No.	Condensed milk		Mix Temp. at freezer ° F.	Brine Temp. ° F.	Overrun readings at minute intervals after brine was shut off									
	Type of condensed milk used	Months in storage at 0° F.			1	2	3	4	5	6	7	8	9	10
1	Fresh	1	36	-4	70	72	75	80	85	88	92	100		
1a	Frozen		36	-2	73	75	77	80	82	85	88	94	100	
2	Fresh	2	36	-4	65	68	75	80	83	90	95	100		
2a	Frozen		36	-2	65	70	75	77	80	85	90	100		
3	Fresh	3	38	-6	60	65	70	70	75	85	90	100		
3a	Frozen		38	-4	60	65	68	75	80	85	86	90	100	
4	Fresh	4	38	-4	67	73	76	81	85	90	95	100		
4a	Frozen		38	-3	70	75	80	85	87	90	95	97	100	
5	Fresh	5	36	4	65	70	76	80	83	90	95	96	100	
5a	Frozen		36	6	67	72	75	77	80	85	88	92	95	100
6	Fresh	6	38	-10	65	70	73	80	84	90	94	95	100	
6a	Frozen		38	-8	66	70	75	79	82	84	89	92	96	100
7	Fresh	7	39	-2	60	65	70	73	78	85	89	95	100	
7a	Frozen		42	-2	56	62	63	65	70	75	82	85	92	100
8	Fresh	8	38	-2	60	65	70	75	80	86	93	100		
8a	Frozen		38	-2	60	65	70	77	82	90	92	97	100	
9	Fresh	9	36	-2	65	70	75	79	83	90	95	100		
9a	Frozen		36	0	60	62	66	70	75	80	85	89	95	100

*Effect of the Use of Frozen Condensed Skimmilk upon Ice Cream Quality*

The ice cream samples were all scored after having been in the hardening room about two weeks as it was believed that this would give most representative conditions. Table 3 shows that there was a noticeable difference in flavor in only three out of the nine comparative pairs of ice cream samples and only one of these samples was made from frozen condensed milk less



GRAPH 1. Overrun readings at minute intervals for 9 batches with each type mix.

than 6 months old. In that case, the sample was criticized for having a "Slight Condensed" flavor. From the seventh month on, the stored condensed skimmilk gave the ice cream samples an off-flavor which was described as "Condensed" or "Lacking in fine flavor." The comparative ice cream samples taken from the ninth month freezings were both given an equal cut in the flavor score.

From the standpoint of body and texture the nine pairs of ice cream samples made from frozen condensed milk were given a slightly lower score than the samples made from fresh condensed skimmilk. In two of the samples there was no difference in score while in the remaining case, the sample containing the fresh condensed skimmilk was given a lower score. "Iciness" to same degree was listed five times, "Coarseness" was listed twice as a

TABLE 3

*Scores and criticisms of ice cream samples obtained from the two types of mixes*

Type of condensed milk in mix	Age condensed milk	Scoring			
		Flavor		Body and texture	
		Criticism	Score	Criticism	Score
Fresh	one month		45		24.0
Frozen			45	coarse	23.5
Fresh	two months		45	sl. fluffy	22.5
Frozen			45	coarse	22.5
Fresh	three mos.		45		24.0
Frozen		sl. cond.	43	sl. icy	23.5
Fresh	four mos.		45	sl. icy	23.0
Frozen			45		23.5
Fresh	five mos.		45		24.0
Frozen			45	sl. icy	23.0
Fresh	six mos.		45		24.0
Frozen			45	icy	23.5
Fresh	seven mos.		45		24.0
Frozen		condensed	43	icy	23.5
Fresh	eight mos.		45		24.0
Frozen		lacks fine flavor	44		24.0
Fresh	nine mos.	lacks fine flavor	44		24.0
Frozen		lacks fine flavor	44	icy	23.5

Perfect Score—Flavor 50, Body and Texture 25.

defect in the samples containing the frozen condensed skim. The defects in no case were serious enough to warrant large cuts and under practical operating conditions such differences would not be noticed by anyone not skilled in judging ice cream.

#### *Effect of the Use of Frozen Condensed Skimmilk upon the Melting Quality of Ice Cream*

The ice cream samples were allowed to melt down at room temperatures; therefore the number of minutes required to collect 100 cc. of melted ice cream would be expected to vary from month to month. This occurred in the case of the samples containing the fresh condensed skimmilk as well as with the samples containing the frozen condensed skimmilk. Average results revealed that under identical conditions, the ice cream samples containing the frozen condensed skimmilk melted down twice as fast as the samples containing the fresh condensed skimmilk.

A possible explanation for this difference may be that the freezing and holding of condensed skimmilk alters the hydrophilic properties of the proteins and the inclusion of such condensed milk in an ice cream mix would alter somewhat the nature and degree of water holding capacity of the proteins in that mix. Sommer (12) states that the hydrated particles form a structure of filaments, with the free water being held in the capillary spaces between the filaments. The gel formed is due to the gelatin and milk proteins present; therefore where the influence of the gelatin was the same in

each case, the gel-forming ability is lessened when the degree of hydration of the milk proteins is lessened.

In every case it was observed that the melted ice cream made from mixes that contained frozen condensed skimmilk showed more foam than the melted ice cream containing the fresh condensed skimmilk.

#### DISCUSSION OF RESULTS

Our experiments have shown that frozen condensed skimmilk, after storage for four or more months, exhibited evidences of gelation upon being melted. This is evidence that some denaturation of the protein has taken place during storage. The poor appearance of the frozen condensed skimmilk upon melting might seem to hinder its use in ice cream making but the appearance of the final mix was not affected at all. When the thawed condensed milk was dumped into a vat as part of a mix, it was easily blended with the other ingredients, and no trouble was experienced in processing the mix.

No significant differences between the two types of mixes were found, as far as the pH and titratable acidity were concerned. Where slight differences were found, these could readily be explained by other factors, not dependent on differences in the condensed milks used.

The difference noted in whipping ability, between the two types of mixes, is small but might be large enough so that the use of frozen condensed skimmilk might not be desirable under certain plant conditions. The number of storage cans and the amount of storage space necessary would be a factor to be considered as to the economy of freezing and storing condensed skimmilk. It would appear that plants having a seasonal surplus of skimmilk would benefit most from such a plan, especially where the condensed product could be made on the premises. Adequate storage space held at a low temperature, is, of course, necessary.

Ice cream made from mixes containing frozen condensed skimmilk scored the same as ice cream made from the mixes made from the fresh condensed skimmilk in a majority of the comparisons made, as far as the flavor was concerned and scored slightly lower in body and texture.

The fact that the ice cream made from the mix that contained frozen condensed skimmilk melted down so much faster than the sample containing fresh condensed skimmilk should not be allowed to discourage any would-be user. It may be noted that these mixes contained only .25 per cent gelatin. If greater melting resistance is desirable increasing the gelatin content would probably produce it.

As frozen condensed skimmilk is usually held at cold storage temperatures (0° F.) and as a 5-gallon can of the frozen product takes from 48 to 60 hours to thaw, it would seem that in many instances frozen milk of this type may be shipped considerable distances without further refrigeration.

Frozen condensed milk has been shipped satisfactorily by the University creamery for distances of 150 miles in hot summer weather on non-refrigerated trucks.

#### SUMMARY AND CONCLUSIONS

1. The storage of frozen condensed skimmilk to be used in ice cream mixes for batch freezing is a satisfactory method of handling surplus skimmilk. A storage period exceeding six months does not appear advisable.

2. After four months in storage at 0° F., condensed skimmilk formed a gel when thawed, and after eight and nine months in storage a "wheying-off" was also observed.

3. No significant differences in pH and titratable acidity between mixes made with frozen condensed skimmilk and fresh condensed skimmilk were recorded.

4. The whipping ability of mixes containing frozen condensed skimmilk was somewhat less than that possessed by mixes containing fresh condensed skimmilk. A slightly longer time was required in the freezer to reach a definite percentage of overrun. No attempts were made to reach a maximum overrun reading.

5. The use of frozen condensed skimmilk in the ice cream mixes caused a lower flavor score in three of the nine trials. The body and texture score was lower in six out of nine cases where frozen condensed skim was used. Neither differences were very significant or consistent.

6. With the gelatin content held constant in each mix, the mix containing frozen condensed skimmilk produced an ice cream that melted down approximately twice as fast as the ice cream made from a mix containing fresh condensed skimmilk.

7. The frozen condensed skimmilk when removed from storage may be shipped considerable distances under comparatively high temperatures with no additional refrigeration necessary.

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# RELATION OF COLOR AND ASCORBIC ACID TO FLAVOR IN MILK FROM INDIVIDUAL COWS<sup>1</sup>

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In 1935, Chilson (4) reported that vitamin C (ascorbic acid) exerted a pronounced influence in preventing the development of oxidized or tallowy flavor in milk when it was added directly to milk. Sharp, Trout and Guthrie (6) later studied this problem and reported that, "There is a positive correlation between the rate of oxidation of ascorbic acid and the rate of development of the oxidized flavor." Brown, Thurston and Dustman (3) found that, "Dry feeding increased the tendency for oxidized flavor to develop in milk, and grazing on fresh pasture decreased this tendency." These latter findings are corroborated by experiments carried out at the New Jersey Agricultural Experiment Station (5). Although it is generally believed that vitamin C in milk cannot be greatly increased by feeding, Brown, Thurston and Dustman (3) found that the feeding of tomato or lemon juice or pure crystalline ascorbic acid greatly decreased the tendency for oxidized flavor to develop even when the cows were on dry feed.

Anderson (1, 2), in collaboration with Harbenbergh and Wilson, has reported that "all available data concerning feeds strongly suggest a relation between carotene and ability to produce milk of good flavor."

In an experiment conducted at the Sussex branch of the New Jersey Agricultural Experiment Station from December 1, 1936, to April 12, 1937, the following factors were studied:

1. The relation of the percentage of fat to yellow color of milk.
2. The relation of yellow color to ascorbic acid in milk.
3. The relation of yellow color to flavor of milk.
4. The relation of ascorbic acid to flavor of milk.

A total of 28 cows consisting of 16 pure bred Guernseys and 12 pure bred Holsteins were used in this study which was carried over a period of approximately four months. The first samples were taken on January 7, 1937, and the last ones on April 29, 1937. Results on samples taken on February 4th and February 11th are not presented in this study since the milks were inadvertently exposed to bright sunlight before reaching the laboratory.

Individual samples from single milkings were taken at weekly intervals and were immediately analyzed for fat by the Babcock method and for

<sup>1</sup> Recently it has been indicated that dehydroascorbic acid shows vitamin C potency. Since the dye-titration method used in this study does not determine the dehydro form the term "ascorbic acid" is used instead of the term "vitamin C."

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yellow color with the "lactochrometer."<sup>2</sup> Each sample was divided into three parts, the last two of which were stored in glass bottles in a refrigerator at about 40° F. The first part of each sample was analyzed for ascorbic acid by the dye-titration method and immediately thereafter was scored for flavor. The second and third parts of each sample were removed from storage on the two succeeding days respectively and were also analyzed for ascorbic acid and scored for flavor.

TABLE 1

*Showing the rank of percentage of fat, flavor, ascorbic acid, and color*

Cow No.	Per cent fat* rank	Flavor rank*		Ascorbic acid rank*		Color* rank
		First day	Third day	First day	Third day	
470 G	6	1	8	9	5	2
428 G	19	2.5	2	14	12	9.5
471 G	8	2.5	4	13	15	7
267 G	7	5	5	4	4	3
467 G	2	5	6.5	21	24	8
479 G	3	5	9.5	3	10	17
179 G	5	7.5	1	8	9	5
465 G	1	7.5	9.5	1	1	1
78 H	23	9	19.5	5	3	20
474 G	12	11.5	6.5	17	19	14
273 G	10	11.5	11.5	2	2	6
422 G	9	11.5	13	10	11	11
165 G	4	11.5	14	6	6	4
92 H	17	14	15	16	14	18
484 G	11	15	21	22	20	13
215 H	13	16.5	3	11	8	15
478 G	15	16.5	16	7	7	12
217 H	28	18	9.5	19	18	24
229 H	18	19.5	17.5	12	13	22
276 H	24	19.5	17.5	20	16.5	19
253 H	20	21	22	18	16.5	23
234 H	27	22	19.5	26	25	28
284 H	25	23	24.5	27	22	26
230 H	26	25	23	23	21	27
286 H	21	25	26	24	26	21
431 G	13	25	27	15	23	9.5
220 H	22	27	24.5	25	27	25
285 G	15	28	28	28	28	16

G—Guernsey; H—Holstein.

\* The ranking of each factor is based on the average for each cow throughout the experiment for that particular factor.

In studying the relation between color, fat, ascorbic acid and flavor statistical analyses of the results were made. The coefficients of correlation were obtained in all cases by Spearman's rank-difference method. A correlation coefficient of .4 or better was considered significant.

<sup>2</sup> The "Lactochrometer" is a modification of the Pfund instrument devised by the Department of Dairy Husbandry of the New Jersey College of Agriculture in cooperation with the American Guernsey Cattle Club for the rapid determination of yellow color in milk from all sources.

TABLE 2  
*Correlations*

Variables	Correlation coefficient
1. Color vs. Percentage of fat	
a. All samples	+ .8702
b. Guernseys	+ .5317
c. Holsteins	+ .7366
2. Color vs. First day ascorbic acid	+ .6847
3. Color vs. First day flavor	
a. All samples	+ .7339
b. Guernseys	+ .4834
c. Holsteins	+ .7229
4. Color vs. Third day flavor	+ .6039
5. First day ascorbic acid vs. First day flavor	+ .6996
6. First day ascorbic acid vs. Third day flavor	+ .5779
7. Third day ascorbic acid vs. Third day flavor	+ .5843

### RESULTS

There was a close relationship between the percentage of fat and the color of milk, Tables 1 and 2. This is shown by a correlation coefficient of +.8702 between these two variables when the data from all 28 cows were used in the computation. When the data were segregated as to breeds the correlation coefficients were +.5317 and +.7366 respectively for Guernseys and Holsteins. This indicates that the percentage of fat had a more positive effect on the color of the Holstein milk than it had on the Guernsey milk.

The correlation coefficient of +.6847 between color and first day ascorbic acid (ascorbic acid determined on the day of the milking) indicates a very significant relationship between these two variables. It is quite possible that both factors are not only closely related to feeds and feeding practices but also may depend alike on certain hereditary factors of the individual cow and on the physiological condition of the cow.

The yellow color of milk is primarily due to the yellow pigment, carotene. A close inspection of the molecular structure and the reactions of carotene reveals that it has reducing properties. It is known that the presence of reducing substances in milk will tend to prevent an oxidizing reaction in the milk. It would be expected, therefore, that carotene would exert a protective action on the flavor of milk. That a close positive relationship exists is shown by correlation coefficients of +.7339 between color and first day flavor and +.6039 between color (first day) and third day flavor (scored after about 56 hours' storage). These two coefficients indicate that, while the relationships between the two factors may be partly coincidental, color or carotene does help to stabilize flavor. This is especially indicated by the correlation between color and flavor after the milk has been in storage for some considerable time.

Ever since the molecular structure of ascorbic acid was discovered, it has been known that the compound has reducing properties. Accordingly

TABLE 3  
*Distribution of flavor scores in ascorbic acid classes*

Ascorbic acid class intervals, mg./l.	Flavor score class intervals								Number cases in each class	Average score for each class	Per cent of cases with score of 23	Per cent of cases with score below 21.5
	23	22.5	22	21.5	21	20.5	20	19.5-12				
30	3	1							4	22.88	75.0	0
27	4		1						5	22.80	80.0	0
24	29	8	2						39	22.85	74.4	0
21	18	24	10	4					100	22.72	62.0	0
18	62	30	14	13	4	1	2		202	22.68	68.3	3.5
15	138	48	36	14	8	1	6	3	237	22.42	51.1	7.2
12	121	92	26	25	11	2	12	6	230	22.20	40.0	12.6
9	92	58	12	7	11		5	10	100	21.73	34.0	28.0
6	34	19	8	5	5		2	5	48	21.45	22.9	25.0
3	11	12	6	1	3		1	2	16	21.34	12.5	37.5
0	2	1										
Totals	496	202	114	69	42	4	28	26	981		50.6	10.2

it can be predicted that ascorbic acid should exert a protective action on the flavor of milk similar to that shown by carotene. A statistical analysis of the data from all 28 cows shows that such a relationship does exist. The correlation coefficient of  $+0.6996$  between first day ascorbic acid and first day flavor is quite significant, while significant coefficients between first day ascorbic acid and third day flavor and between third day ascorbic acid and third day flavor indicate that ascorbic acid helps to stabilize flavor. This relationship is further shown in Table 3. In making this table the ascorbic acid values were divided into three-milligram intervals; the flavor scores were divided into 0.5-point intervals. In plotting the distribution, only data from single samples were used; no averages appear in this table. There was a total of 981 samples on which the flavor score and ascorbic acid content were obtained. An inspection of the average flavor score for each ascorbic acid class interval reveals that with a decrease in the amount of ascorbic acid a decrease in the flavor score occurs. A further inspection of the data reveals that an apparent critical point in the relation of ascorbic acid to good flavor lies somewhere between 15 and 18 milligrams of ascorbic acid per liter of milk.

#### CONCLUSIONS

From the data presented in this paper it can be concluded that in the milk from individual cows:

1. There is a close relation between percentage of fat and yellow color.
2. There is a significant association of yellow color and ascorbic acid.
3. High carotene and high ascorbic acid are coincidental to and help to preserve good flavor in milk.

It is recommended, therefore, that special efforts be made to preserve the carotene in roughages and that winter feeding practices be followed so as to maintain a high level of yellow color in the milk.

It is further recommended that special efforts be made to preserve the ascorbic acid in milk by subjecting the milk to a minimum amount of aeration, by prevention of contamination with such metals as copper, iron and nickel, and by protection of the milk from sunlight.

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# RELATION BETWEEN LACTOSE AND ASH CONTENT OF THE MILK OF DIFFERENT MAMMALS

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The freezing-point depressions of the milk and blood of cows are essentially the same. The freezing-point depression of the milk is almost entirely due to lactose and salts. Milk from diseased udders or from cows in ad-

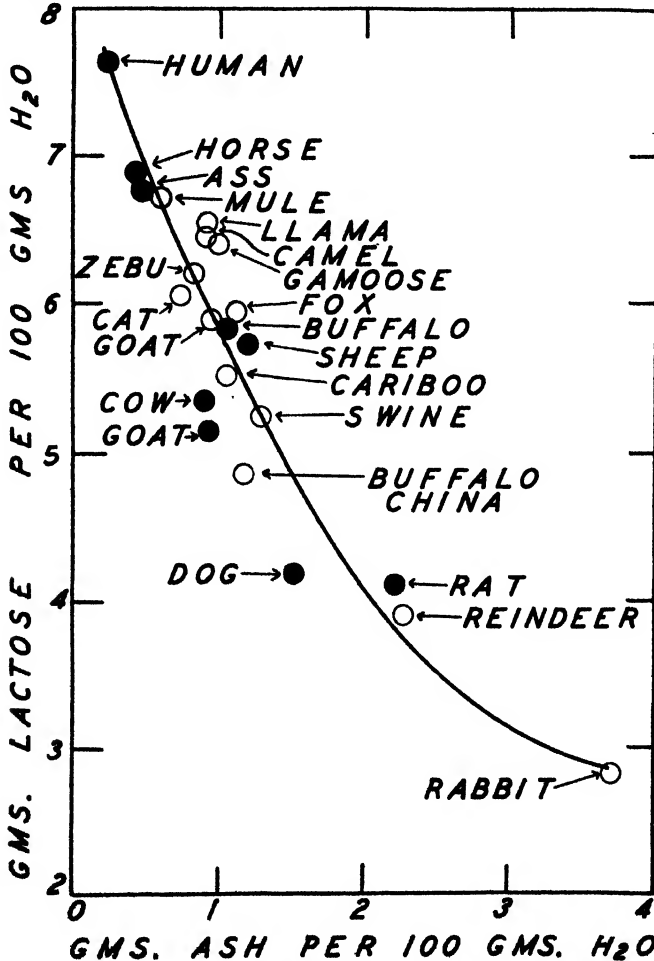


FIG. 1. Relation between lactose and ash content of the milk of different mammals when calculated to the water basis. Circles represent few data available; dots represent more extensive data.

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vanced lactation is relatively low in lactose, but this is compensated for by a higher salt content, the freezing-point depression remaining essentially unchanged (1, 2, 3). These alterations, particularly the increase in chloride content (4), are used for detecting abnormal milk. Since the blood and milk are essentially in osmotic equilibrium and the freezing point of the blood of the different mammals is much the same, one would expect the osmotic pressures of their milks to be about the same.

The milk of different mammals for which apparently reliable data on composition could be found ranged in average values for water from about 65 to 88 per cent; for ash from 0.23 to 2.5 per cent; and in lactose from 2.0 to 6.7 per cent. These wide variations are brought into a definite relationship to each other when calculated on the basis of the amount of water in the milk. This relation is shown in Figure 1.

The data were taken from compilations (5) and many individual articles. Data were not included when it was obvious that either the milk was abnormal or the values were in error. Some variation was to be expected because of the differences in analytical methods. The insolubility of some of the ash constituents, the presence of simple organic compounds, and the variation in the percentage composition of the ash constituents might exert an influence, but these variations (5) apparently affect the general relationship to only a minor degree.

The data found for the aquatic mammals, the elephant and the hippopotamus were not included because of inconsistencies. These and the hippopotamus were probably due to the difficulties in obtaining normal, representative samples of milk.

The inverse relationship between lactose and ash constituents has long been recognized in the variations occurring in the milk of the cow. Figure 1 shows that in general the same inverse relationship holds when applied to the milk of different mammals when the calculations are made on the basis of water content. The curve serves as a rough criterion for the reliability of lactose and ash values for milk.

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# A METHOD OF COUNTING VIABLE BACTERIA IN MILK BY MEANS OF THE MICROSCOPE<sup>1</sup>

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## INTRODUCTION

The value to the dairy industry of a microscopic method which allows distinction between dead and living bacterial cells needs no emphasis. Heretofore, the difficulties in the way of such a method seemed insurmountable. The two attempts we were able to find in the literature are those of Beattie (1) and Nadelin (13) which are really applications of Proca's method (14) and of its modification by Kayser (7). Failure of these attempts was to be expected because of defects inherent in the methods of Proca and Kayser. Knaysi's criticism (8) of these methods has been confirmed again in the present investigation.

## PRELIMINARY EXPERIMENTS

Before the writers undertook the present investigation, they were fully aware of the magnitude of the task. They found it necessary to approach the problem with open minds and to avail themselves of the published experiences of other investigators who concerned themselves with the problem of microscopically distinguishing dead from living cells. Accordingly, we tried, but without success, Proca's method as used by Beattie and in the form of Kayser's modification. We were also unsuccessful when we used Růžička's method (15), Benians' method (2) and its modification by Henrici (6), Drobot'ko's method (3), and an adaptation of the recent one suggested by Luyet (11). In the latter case, we attempted several other modifications of our own, but got nowhere. The only application which seemed to hold promise for us was that of "vital staining" with basic dyes as used by the senior author (1935).

## *Difficulties of Application*

It has been shown by Knaysi (8) that the slightest shade of staining of the cytoplasm indicates death of the cell, while the cytoplasm of a viable cell is perfectly colorless. With basic dyes the process of staining is instantaneous and is not dependent on autolysis; on the contrary, autolysis reduces stainability with basic dyes. Our problem was, therefore, to make such a method applicable to milk. In this case it is not sufficient for the

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method to be scientifically correct; it must be, in addition, simple, practical and, if we may say so, "fool-proof," before it can be suitable for the routine work of milk control. It was, therefore, necessary to make several changes in the technique used by the senior author, which consisted in mixing measured volumes of culture and neutral red containing gelatin, and counting in a Petroff-Hauser chamber after cooling the chamber in an icebox to cause hardening of the gelatin.

In the first place, the Petroff-Hauser chamber is expensive; secondly, room temperature is often too warm and one would have to work in a cool room to keep the gelatin hard; and thirdly, neutral red, which was selected for the theoretical study on account of its low characteristic potential, is not the best dye to use because of its color, its sensitivity to pH and its ability to stain fat with a yellow tinge. Moreover, milk contains an immense number of minute fat droplets which, when singly or in pairs are easily confusable with viable coccus cells.

#### *Development of the Technique*

Knaysi and Naghski (9), in an unpublished work, had substituted for the counting chamber the following technique: 0.01 cc. of the milk-gelatin mixture is placed on a clean slide and a clean cover glass is dropped over the droplet formed. The droplet is thus flattened into a film between the slide and the cover glass, which occupies an area equal to that of the face of the cover glass. This area, being known, a factor can be computed. In our work, we have adhered to this technique; however, in order to keep the factor down, we selected the smallest cover glass available to us, a square one, with 1.5 cm. to the edge. This gives, when used with an oil immersion objective and a  $10\times$  ocular ( $10\times 97$  combination), a factor of about  $2.25 \times 10^6$ . Using a  $6\times$  ocular, the factor is reduced to about  $1.35 \times 10^6$ . In a few of our later experiments we cut our cover glasses down to  $1.28 \times 1.28$  cm., thus having a factor of  $1 \times 10^6$  when the  $6\times 97$  combination is used.

The next step was to modify the hardening agent. For this purpose we made several determinations of the "melting" and "solidification" points under practical laboratory conditions, of some agar and gelatin gels and of mixtures of the two when added to milk. On the basis of the results obtained, we picked a mixture which, when added to the milk, gives a percentage of 0.5 per cent agar and 2 per cent gelatin. Milk containing these percentages of agar and gelatin usually hardens in a few minutes at  $23^{\circ}$ - $24^{\circ}$  C. and once hard, has to be heated to about  $70^{\circ}$  C. before it melts.

Instead of neutral red, we used Nile blue sulfate, alone or mixed with methylene blue. The blue color gives a greater contrast, and the fat droplets take up an orange-red color which helps to set them apart. Therefore, when using such a mixture, the dead bacteria appear blue, the viable ones remain colorless, and the fat droplets assume various shades of red.

*The Removal of Fat*

Milk contains a large number of fat droplets many of which are within the size range of bacterial cells. It is these droplets that are most troublesome because as it was pointed out above, they can frequently be confused with viable coccus cells; attempt was therefore made to remove the fat. Unfortunately, it was found that the smallest fat droplets cannot be removed by ordinary centrifuging, and we found it necessary to think of removing the fat chemically without killing the bacteria in the milk. For that purpose, we experimented with several fat solvents, including hydrocarbons of the methane series.

The experiments were carried out as follows: Sterile skimmilk was inoculated with a given organism and incubated at 37° C.; when sufficient growth had taken place, one cc. was measured into a water blank for plating, and the fat solvent was then added to the rest of the culture and the tube shaken vigorously for about two minutes. The shaken mixture was then allowed to stand for five minutes, after which it was centrifuged to remove the solvent droplets distributed throughout the tube. In the meantime, plating of the cc. first measured was carried out. After centrifuging, the culture treated with the fat solvent was also plated. Tables 1 and 2 contain the

TABLE 1  
*Effect of shaking with pentane and hexane*

Organism	Plate counts		
	Before treatment	After treatment with pentane	After treatment with hexane
<i>Streptococcus lactis</i>	182,000,000	165,000,000	209,000,000
<i>Streptococcus mastitidis</i>	536,000,000	504,000,000	302,000,000
<i>Escherichia coli</i>	221,000,000	260,000	27,000,000
<i>Proteus vulgaris</i>	153,000,000	20,000	8,000,000
Unidentified organism	319,000,000	252,000,000	274,000,000

TABLE 2  
*Effect of shaking with hexane and heptane*

Organism	Plate counts		
	Before treatment	After treatment with hexane	After treatment with heptane
<i>Streptococcus lactis</i>	61,000,000	58,000,000	59,000,000
<i>Escherichia coli</i>	39,200,000	36,000,000	67,000,000
<i>Aerobacter aerogenes</i>	4,800,000	1,900,000	5,400,000
<i>Proteus vulgaris</i>	61,000,000	27,000,000	109,000,000
Unidentified organism	33,000,000	7,000,000	11,000,000

results obtained with pentane, hexane and heptane. These results are in agreement with those of Hall (4), Walbum (17), and Morel, Rochaix and Mathais (12) regarding the toxicity of hydrocarbons.

Since heptane showed practically no toxic power toward the organisms employed, it was adopted for use in this work whenever fat removal was desired. However, when the final technique was developed, it was found that fat removal can be dispensed with.

We wish also to report at this point certain experiments in which we sought to remove the milk fat by agglutination and subsequent centrifuging. In an unpublished work, Krukovski and Sharp (10), of this department, discovered in milk an agglutinin for the fat droplets. On account of a specific protein film surrounding fat droplets in milk, we sought to develop agglutinins for these droplets, by injecting cream, washed free from casein, into guinea pigs. Two guinea pigs received daily intraperitoneal injections with this washed cream. After six days, the pigs were allowed to rest for six more days, after which they were bled from the heart and their serum added to milk in various concentrations. Definite agglutination of the fat droplets was observed in dilutions of  $10^{-2}$  and below at  $35^{\circ}$  C. However, the process was found slow and unsuitable, and the removal of fat incomplete. We wish here to express our thanks to Dr. F. R. Smith for his technical help in this phase of the work.

#### FINAL PROCEDURE

After considerable testing and re-testing of steps and materials and the working out of many details, we standardized our procedure as follows:

1. One cc. of the well mixed sample is measured into each of two clean test tubes, A and B.
2. To each test tube is added 0.5 cc. of a dye mixture containing equal parts of 0.2 per cent methylene blue and 0.2 per cent Nile blue sulfate, and the contents of each tube mixed well. The final concentration of each dye in the completed preparation will be 0.025 per cent. Table 3 shows that in

TABLE 3

*Effect of the dyes on the growth of milk cultures*

10 cc. of culture was diluted with 5 cc. of sterile distilled water or with 5 cc. of a dye solution containing 0.1 per cent of Nile blue sulfate and 0.1 per cent of methylene blue. Plate counts were then made at intervals.

Time in min.	Streptococcus lactis plate count per cc. when diluted with		Aerobacter aerogenes plate count when diluted with	
	Sterile water	Dye solution	Sterile water	Dye solution
0	31,000,000	35,000,000	29,000,000	20,000,000
10	46,000,000	29,000,000	24,000,000	17,000,000
20	35,000,000	28,000,000	31,000,000	24,000,000
30	42,000,000	24,000,000	29,000,000	20,000,000
40	29,000,000	30,000,000	33,000,000	31,000,000
50	32,000,000	32,000,000	32,000,000	21,000,000
60	42,000,000	31,000,000	33,000,000	29,000,000
80	44,000,000	34,000,000	49,000,000	42,000,000
120			76,000,000	62,000,000

such a concentration these dyes are not toxic to *Streptococcus lactis* and *Aerobacter aerogenes*.

3. To one of the tubes, say tube B, add a drop of 0.7–0.8 N NaOH and mix. Tube A receives nothing.

4. To each tube add 0.5 cc. of the melted and tempered hardening agent which is an agar-gelatin mixture containing 2.0 per cent agar and 8.0 per cent gelatin and mix the contents of each tube. Before this is done, the tubes are placed for a few moments in the water bath alongside the melted agar-gelatin mixture. The temperature of the bath should be about 45°–50° C. This prevents the instantaneous hardening of the mixture. The hardening agent is now present in the concentration referred to above, namely, 0.5 per cent agar and 2.0 per cent gelatin.

5. With a Breed pipette, measure out 0.01 cc. from tube A and place on the center of the right half of a clean glass slide, and immediately cover the droplet thus formed with a clean square cover glass 1.28 × 1.28 cm. The droplet spreads and forms a film occupying the space between the cover glass and the slide.

Do the same with tube B, placing the 0.01 cc. droplet on the center of the left half of the slide, etc.

6. Let the preparation stand or place it in the icebox for about two minutes so that the films harden.

7. Count the blue-stained bacterial cells in a certain number of fields of each film and calculate the bacterial content of each per cc. With a 6 × 97 combination, and using 1.28 × 1.28 cm. cover glass, the factor is about 1,000,000.

The stained cells from tube A represent the dead cells present originally in the milk. The stained cells from tube B represent the total bacterial content of the milk. The difference represents the viable cells present in the milk.

With ordinary market milk we found it in general unnecessary to remove the fat. If it is desired to remove the fat, the milk sample should be warmed to 35° C. to liquefy the fat; heptane is then added and shaken vigorously for 3 to 5 minutes and centrifuged. A Babcock hand or electrically driven centrifuge is sufficient. The hydrocarbon-fat layer is then removed. A convenient way of removing this layer is by suction with a capillary glass tube attached to a water pump.

Even after this treatment, a certain number of the minute fat droplets remain. These show only a trace of orange color and could be confused with colorless cocci. This is one of the reasons why we finally modified our technique to the double-counting procedure outlined above. Moreover, we find it hard on the eyes to hunt for colorless cells, and easy to overlook many of them. This explains the low counts of Table 4. In the above procedure all the cells counted are stained deep blue against an almost colorless back-

TABLE 4

*A comparison of counts obtained by the single count technique (living cells unstained) and by the petri plate method*

Experiment No.	Culture employed	Dead cells $\times 10^6$	Group count $\times 10^6$	Total cell count $\times 10^6$	Plate count $\times 10^6$
5 . . .	<i>Streptococcus thermophilus</i>	0	17.8	28.1	40.0
8 . . .	<i>Streptococcus fecalis</i>	11.1	28.6	43.0	280.0
16 . . .	<i>Aerobacter aerogenes</i>	0	10.6	14.0	66.0
20 . . .	Unidentified culture	0	10.8	14.0	71.0
27 . . .	<i>Aerobacter aerogenes</i>	4.7	11.7	20.2	36.0
40 . . .	<i>Aerobacter aerogenes</i>	.5	6.7	21.4	20.0
48 . . .	<i>Aerobacter aerogenes</i>	4.2	6.8	11.7	27.8
54 . . .	<i>Aerobacter aerogenes</i>	12.8	13.7	15.5	35.0
59 . . .	<i>Aerobacter aerogenes</i>	7.2	6.3	8.1	49.0

ground. Even the presence of the fat droplets does not materially interfere with the visibility, mainly because in this method the fat droplets are mostly distributed throughout the film, and do not accumulate into a layer on the top of the film.

#### *Calculation of the Factor*

The procedure followed in computing the factor is similar to the one used in Breed's method. To illustrate, we propose to find the factor when  $1.28 \times 1.28$  cm. cover glass and when a combination is used that gives a field diameter of  $160 \mu$ . The area of the field will be  $\pi r^2$  or  $3.1416 \times 0.008 \times 0.008 = 3.1416 \times 0.000064$  sq. cm. or about 0.0002 sq. cm.

The area of the preparation is  $1.28 \times 1.28 = 1.64$  sq. cm., which is equivalent to  $1.64 : 0.0002 = 8200$  fields, and contains 0.005 cc. of milk. The volume under each field would hold  $.005 : 8200 = \frac{5}{8,200,000}$  cc. milk. The factor would, therefore, be 1,640,000. Roughly with the same combination, the factor is nearly three times that of the Breed smears.

#### APPLICATION OF THE METHOD

The method was applied to milk cultures of a number of organisms and to pasteurized market milk that in some cases had been allowed to incubate until it developed a high count. The results are recorded in Tables 5 to 9. For the sake of comparison, petri plate counts were included in each experiment, and are recorded in the tables. Although counts by our method did

TABLE 5

*A comparison of differential microscopic counts and plate counts  
Streptococcus thermophilus*

Experiment	Dead cells $\times 10^6$	Total count		Living cells $\times 10^{**}$	Plate count $\times 10^6$
		Groups $\times 10^6$	Individuals $\times 10^6$		
1	4.5	9.0	18.0	13.5	8.0
2	3.0	5.8	11.7	8.7	Spoiled
3	2.0	6.7	21.1	19.1	Spoiled
4	7.0	4.8	13.5	6.5	4.6
5	5.1	5.2	10.0	4.9	3.9
6	2.7	4.0	10.7	8.0	5.6
7	0.9	4.2	13.2	12.3	9.0
8	0.55	3.15	9.7	9.15	10.0
9	0.70	8.0	19.1	17.4	13.0
10	0.45	4.0	10.3	9.85	7.3
11	0.23	1.5	3.8	3.57	2.9
12	0.34	1.25	4.5	4.16	3.6
13	0.22	1.12	3.37	3.15	1.2
14	0.7	5.8	27.7	27.0	17.0
15	1.0	6.0	36.45	35.45	13.0
16	1.13	10.1	23.3	22.17	41.0
17	0.34	4.5	10.7	10.36	7.6
18	0.78	5.1	12.8	12.02	9.0
19	0.56	13.2	34.9	34.34	24.0
20	0.45	3.3	9.4	8.95	6.0
21	0.45	8.4	23.1	22.65	15.0
22	0.45	8.7	24.1	23.65	20.0
23	1.10	9.2	25.4	24.3	12.0
24	0.6	7.3	24.6	24.0	20.0
25	0.45	11.2	31.0	30.55	25.0
26	0.6	6.1	20.25	19.65	13.0
27	0.78	7.9	28.7	27.92	12.0
28	0.35	2.0	7.1	6.75	4.1
29	1.90	13.3	41.2	39.3	27.2
30	0.90	5.1	19.5	18.6	12.6
31	0.23	1.79	6.2	5.97	2.0
32	0.67	1.69	4.9	4.23	1.8
33	0.30	1.24	3.3	3.0	1.4
34	0.45	1.35	6.5	6.05	Spoiled
35	0.56	1.8	5.4	4.84	7.0
36	0.67	3.6	8.3	7.63	7.7

\* Total individuals minus dead cells.

not include viable groups and cannot strictly be compared to the plate counts, yet we find the great majority of the plate counts occupying the range between the total group and viable individual counts of our method. Of interest are the experiments made on the pasteurized milk. The majority of the plate counts fall below the total group count, which is often higher than the number of viable cells. These results are what one would expect from pasteurized milk.

#### *Stainability of Cells Killed by Pasteurization*

The stainability of cells killed by pasteurization has been investigated by a number of workers. Hastings and Davenport (5) concluded that the

TABLE 6

*A comparison of differential microscopic counts and plate counts  
Streptococcus lactis*

Experiment	Dead cells $\times 10^6$	Total count		Living cells $\times 10^{**}$	Plate count $\times 10^6$
		Groups $\times 10^6$	Individuals $\times 10^6$		
1 .....	0.70	21.6	82.3	81.6	37.0
2 .....	0.56	17.3	53.1	52.54	35.0
3 .....	1.2	13.3	38.0	36.8	29.0
4 .....	1.2	15.3	48.1	46.9	36.0
5 .....	0.75	13.5	32.2	31.45	22.0
6 .....	0.9	10.6	21.8	20.9	25.0
7 .....	0.9	8.1	21.2	20.3	20.0
8 .....	0.45	9.5	20.9	20.45	27.0
9 .....	0.15	9.6	28.1	27.95	30.0
10 .....	0.56	2.3	5.1	4.54	4.2
11 .....	0.9	6.4	12.8	11.9	10.0
12 .....	0.25	3.3	7.2	6.95	6.5
13 .....	0.34	1.13	2.25	1.91	2.8
14 .....	1.4	7.1	17.1	15.7	17.0
15 .....	2.0	3.0	4.7	2.7	Spoiled
16 .....	1.1*	2.4	3.4	2.3	3.0
17 .....	1.35	4.15	8.0	6.65	8.0
18 .....	0.9	5.8	14.3	13.4	13.5
19 .....	0.67	8.3	15.2	14.5	13.0
20 .....	0.45	6.5	13.7	13.25	12.5
21 .....	1.0	1.3	2.7	1.7	.9
22 .....	0.23	1.24	1.69	1.46	1.0
23 .....	0.34	0.79	1.35	1.0	.8
24 .....	0.11	0.61	1.05	0.94	1.0
25 .....	0.10	4.5	14.6	14.5	15.0
26 .....	1.1	5.3	18.35	17.25	8.2
27 .....	0.23	1.47	2.9	2.67	3.9
28 .....	0.56	3.15	7.9	7.34	5.3
29 .....	0.67	1.45	1.8	1.13	0.9
30 .....	0.34	1.8	3.5	3.16	2.8
31 .....	0.11	1.1	1.8	1.69	2.6
32 .....	0.11	2.15	3.8	3.69	5.6
33 .....	0.56	1.8	3.7	3.14	2.2
34 .....	0.45	2.0	3.05	2.6	2.5

\* Total individuals minus dead cells.

number of bacteria demonstrable in milk after pasteurization by Breed's method varies from 3 to 83 per cent of the number demonstrated in the same milk before pasteurization, and depends considerably on the type of bacteria present. The results obtained by Ward and Myers (18) with pasteurized milk are similar, but these authors did not study the effect of the type of organisms killed by pasteurization. The conclusion drawn by Hastings and Davenport was that the direct microscopic method does not indicate the quality of the milk before pasteurization. On the other hand, Ward and Myers conclude "that insufficient numbers of dead bacteria remain visible after pasteurization to impair the usefulness of direct microscopic counts made on pasteurized milk." The latter authors, therefore,

TABLE 7

*A comparison of differential microscopic counts and plate counts  
Bacillus subtilis*

Experiment	Dead count total cells $\times 10^6$	Total count		Living cells $\times 10^{**}$	Plate count $\times 10^6$
		Groups $\times 10^6$	Individuals $\times 10^6$		
1.	0.65	1.13	3.15	2.50	0.3
2..	0.23	0.56	2.25	2.02	0.4
3.	0.45	1.2	3.4	2.95	1.4
4.	0.80	2.5	7.3	6.5	2.5
5.	0.12	.81	2.0	1.88	1.4
6.	0.34	1.8	4.4	4.06	3.1
7.	0.23	1.35	2.9	2.67	1.8
8.	0.12	4.0	8.2	8.08	5.0
9.	0.12	2.6	4.8	4.68	2.9
10	0.23	2.4	3.8	3.57	5.1
11	0.34	1.8	3.8	3.46	2.9

\* Total individuals minus dead cells.

TABLE 8

*A comparison of differential microscopic counts and plate counts  
Staphylococcus albus*

Experiment	Dead cells $\times 10^6$	Total count		Living cells $\times 10^{**}$	Plate count $\times 10^6$
		Groups $\times 10^6$	Individuals $\times 10^6$		
1	0.45	4.6	8.4	7.95	4.6
2	0.25	3.15	4.6	4.35	3.0
3			Spoiled		1.2
4.	1.7	7.3	20.7	19.0	9.1
5.	0	1.0	2.6	2.6	2.3
6	0.11	4.05	8.9	8.79	10.3
7.	0.34	1.8	5.3	4.96	3.6
8†	0.4	2.5	9.35	8.95	8.1
9†	0.14	2.15	6.20	6.06	6.3
10†.....	0.2	3.9	7.8	7.6	10.5

\* Total individuals minus dead cells.

† Counts made on whole milk without fat removal and using the  $6\times$  ocular.

consider counts obtained by the direct microscopic method to represent viable cells.

While we have not yet done sufficient work on this point to justify our taking a stand, we wish to report some preliminary results we obtained with milk and with pure cultures of *Streptococcus lactis*. The milk, or the culture was held at  $63^{\circ}$ – $65^{\circ}$  C. for 30 minutes, and immediately afterwards a count was made by our method and, in the case of milk, by the method of Breed, using Newman's solution no. 2 for staining. The results are recorded in Tables 10 and 11. These results show that the pasteurization of milk does not render the killed bacteria invisible when our method is used. On the other hand, we find confirmation of the earlier reports that pasteurization

TABLE 9  
Comparison of differential microscopic counts and plate counts  
Commercial pasteurized milk

Experiment	Dead count total cells	Total count		Living cells $\times 10^{10}$ <sup>1</sup>	Plate count $\times 10^6$
		Groups $\times 10^6$	Individuals $\times 10^6$		
1†	1.0	1.57	3.4	2.4	2.42
2†	1.0	1.01	1.35	0.35	.011
3†	1.125	1.8	3.4	2.275	0.80
4†	1.8	2.0	2.47	0.67	0.49
5†	1.35	1.7	1.90	0.55	0.47
6	1.1	1.8	2.0	0.9	0.61
7	1.57	16.2	25.0	23.4	36.0
8	8.65	9.35	12.4	3.75	2.0
9	2.25	12.25	18.75	17.5	13.0
10	0.34	0.27	0.42	0.08	0.01
11	0.21	0.54	1.01	0.8	0.30
12†	0.45	3.65	6.2	5.75	6.1
13†	1.75	24.0	33.0	31.25	13.6
14†	0.2	2.5	3.5	3.3	2.2
15	1.35	15.5	23.0	21.65	11.7
16	0.34	1.6	2.0	1.66	1.3
17†	0.135	3.0	4.8	4.665	9.0
18†	0.34	0.405	0.54	0.20	0.15
19†	0	0.135	0.135	0.135	< 0.01
20†	0	5.4	8.8	8.8	0.02
21†	0.27	3.0	3.8	3.53	0.01
22†	2.07	4.9	6.9	4.83	0.33
23†	0.27	4.07	6.1	5.83	5.1

\* Total individuals minus dead cells.

† Fat removal with heptane.

‡ Counted with 6 $\times$  ocular.

zation does render a certain percentage of the killed bacteria unstainable when the standard direct microscopic method is used.

This preliminary work is sufficient to prove that the bacteria made invisible by pasteurization do not vanish, but that their stainability is often sufficiently reduced so that they do not show by methods requiring decolorization or when stains containing decolorizing agents are used. We did notice by our method, that bacteria killed by heat in aqueous suspensions

TABLE 10  
A comparison of counts of *Streptococcus lactis* before and immediately after heating  
to 63°–65° C. for 30 minutes

Culture number	Before heating		After heating	
	Groups	Individuals	Groups	Individuals
1	1,200,000	5,250,000	1,200,000	4,050,000
2	1,600,000	5,400,000	1,070,000	4,200,000
3	1,350,000	2,800,000	1,200,000	3,200,000
4	1,900,000	4,050,000	1,750,000	4,700,000
5	810,000	3,500,000	675,000	1,500,000
6	400,000	1,900,000	810,000	2,300,000

TABLE 11

*Effect of heating the milk to 63°–65° C. on microscopic counts made by Breed's method and by the cover-slip technique. Both counts are made on the same sample of milk*

Sample number	Before heating		After heating	
	Groups	Individuals	Groups	Individuals
<i>Breed Smears</i>				
1 . . . . .	2,800,000	7,000,000	1,700,000	4,100,000
2 . . . . .	1,800,000	6,200,000	1,800,000	3,500,000
3 . . . . .	13,000,000	42,000,000	9,200,000	20,500,000
4 . . . . .	5,400,000	7,000,000	4,300,000	5,200,000
5 . . . . .	1,050,000	3,600,000	600,000	1,250,000
<i>Cover-slip preparations</i>				
1 . . . . .	4,450,000	12,200,000	5,000,000	11,600,000
2 . . . . .	2,100,000	4,750,000	2,100,000	3,800,000
3 . . . . .	17,000,000	31,600,000	19,500,000	24,700,000
4 . . . . .	5,100,000	18,700,000	6,250,000	16,000,000
5 . . . . .	2,200,000	4,750,000	2,000,000	5,050,000

are not as deeply stained as those killed by chemicals or by dry heat. It seems probable that this is due to the loss of the water-soluble, stainable materials from the cell, such as nucleic acid, lipoids, etc.

## DISCUSSION

The soundness of the principles upon which the present method is based has been retested by us by observing the behavior toward our dye mixture, of viable cells and of cells killed by various means, including the quick cooling method of Sherman and Cameron (16). The preparations present an almost colorless background with the dead cells deep blue. The alkali accomplishes two purposes: In the first place, it makes the dye mixture sufficiently toxic to kill the viable cells; secondly, it acts as a mordant, intensifying the staining of these cells. This apparently dual rôle of the alkali is in reality brought about by the same mechanism, namely, the increased affinity between the cell substance and the dyes.

There is one objection to the method that has been already expressed to us, and that is the magnitude of the factor. The best we could do was to use  $1.28 \times 1.28$  cm. cover glasses with the  $6 \times 97$  combination. This gives us a factor of  $1 \times 10^6$ , which many people consider too large. We are not of this opinion. It is true that the high figures necessary in all direct microscopic methods constitute the limitation of these methods, yet we believe that the degree of contrast obtained in our technique, and the excellent distribution of the cells more than compensate for the relative increase in the factor. If 50 fields of a preparation be explored, a single cell encountered would be equivalent to 20,000 cells per cc. In many states this figure is not

too high for ordinary market milk. In any case, we are not suggesting our method as a substitute for the petri plate.

### *Other Possible Uses*

This method may be used, not only in grading raw and pasteurized milk, with a clue to the history of the latter, but it can also be used in determining viable cells in butter cultures, and may also be modified for use in the bacteriological control of other dairy products.

### SUMMARY

A direct microscopic method for distinguishing dead from viable bacterial cells in milk is reported. The method is based on the principles of vital staining. One cc. of the milk to be examined is mixed with 0.5 cc. of a dye mixture containing 0.1 per cent methylene blue and 0.1 per cent Nile blue sulfate, and with 0.5 cc. of a gelatin-agar mixture containing 8 per cent gelatin and 2 per cent agar. 0.01 cc. of this mixture is placed on a clean glass slide and covered with a small cover glass ( $1.28 \times 1.28$  cm.). The stained cells are then counted and they represent the dead cells already present in the milk. To a similar mixture one drop of 0.7 to 0.8 normal NaOH is added. This causes staining of all the cells present in the milk. The difference between the two counts represents the number of viable cells present.

The object of the agar-gelatin mixture is to solidify the preparation, keeping the bacteria in position. The factor is calculated from a knowledge of the amount of milk present in the preparation and the area of the cover glass.

When desirable, fat can be removed by shaking the milk with heptane followed by moderate centrifuging.

A number of tables are included showing the results obtained when the method is applied to pure cultures and to pasteurized milk. The counts obtained are also compared to petri plate counts made on the same material.

The effect of pasteurization on the stainability of bacteria in milk is studied and briefly discussed.

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## COD-LIVER OIL TOLERANCE IN CALVES

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A previous extensive report from this laboratory by Madsen, McCay and Maynard (1) has described the production of muscle dystrophy in guinea pigs, rabbits, sheep and goats on rations containing cod-liver oil. While the injury occurred more regularly and with greater severity with synthetic diets containing the oil, it was clearly shown that rations of natural foods were made dystrophic by oil addition. The data indicated that the injurious factor resided primarily in the saponifiable fraction and no evidence was obtained that the vitamins present in the oil were in any way responsible for the lesions. These findings were extended in further studies with guinea pigs and rabbits by Madsen (2) at Columbia University, as a result of which it became evident that the severity of injury was influenced by certain dietary interrelationships.

During the past two years the problem has continued to receive attention in this laboratory, primarily in studies with guinea pigs, with the objects of identifying and eliminating the specific harmful factor and of learning more about the dietary interrelationships involved. These experiments are being reported elsewhere (3). During the same period, however, studies have been in progress with calves to ascertain whether this species is susceptible to the injury when cod-liver oil is used as a supplement to commonly fed rations. Such studies seemed particularly called for in view of brief reports by Slagsvold (4) and by Agduhr (5, 6, 7) of such an injury and in view of the increasing practice of using cod-liver oil or its products as vitamin supplements in calf feeding. Our experiments are described in the present paper.

The general procedure in these experiments has been to add graded levels of the oil to the rations of calves, beginning shortly after birth and continuing for periods ranging from six to nine months. The animals were then slaughtered for gross and microscopic examination of the organs and tissues. The same oil levels were used as had been employed in the previous studies with sheep and goats being reared on pasture, namely, 0.1, 0.35, and 0.7 gram per kilo live weight. The oil was the same product, designated as "animal grade," used in the previous studies, and stated to contain in excess of 1000 A. D. M. A. units of vitamin A and in excess of 150 A. D. M. A. units of vitamin D. Trials with guinea pigs had shown that a U. S. P. grade of oil was equally injurious. Two experiments were conducted with the calves. Since the rations employed and the procedures were somewhat different, the experiments are described separately.

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## FIRST EXPERIMENT

Calves were purchased from nearby farms and brought to the University shortly after birth. After a brief period during which they were fed whole milk, given anti-scours serum and kept under veterinary observation, those which appeared disease-free and otherwise thrifty were placed on experiment. They were kept in individual pens. All of the animals were fed substantially alike with the exception of the cod-liver oil supplement. They received whole milk until three weeks of age, and then were gradually changed to skim milk which was fed in accordance with their appetite with a maximum intake of 12 pounds per day. The feeding of skim milk was discontinued at four and one-half months of age. The calves were given clover-timothy hay of good quality and the following grain mixture:

600 pounds wheat bran  
500 pounds hominy feed  
460 pounds ground oats  
200 pounds linseed oil meal  
200 pounds molasses  
20 pounds bone meal  
20 pounds salt

The calves were fed the hay *ad libitum*, and allowed all the grain they would eat up to a maximum of three pounds per head per day. The oil was given as a drench beginning at two weeks of age. Three animals received the highest level, two the intermediate level, three the lowest level, and two received 0.1 gram per kilo of a "reinforced" cod-liver oil<sup>1</sup> frequently used as a vitamin supplement for calves. Pertinent data regarding the calves are given in Table 1. The oil intakes were adjusted weekly in accordance with the weights of the animals. The amount of oil fed the calves receiving the highest level, however, was not increased beyond 200 grams per day, and in the case of the 0.35-gram level, not beyond 100 grams per day.

The animals were observed daily for any indications of unthriftiness or of more specific troubles. Weights were taken weekly. No digestive or other disturbances were noted on beginning the oil feeding. All of the animals grew well and remained in good condition during the first six weeks. Shortly thereafter, however, No. 9 started to lose weight and failed rapidly. The trouble was diagnosed as pneumonia and treatment was begun accordingly. The calf continued to fail and died after 62 days on the experiment. The post-mortem findings are discussed later.

All of the other animals continued to grow regularly and showed no evidence of unthriftiness. At 26 weeks one animal from each group was slaughtered for post-mortem examination and the remaining animals were

<sup>1</sup> This product was guaranteed to contain 400 U. S. P. units of vitamin D and 3000 U. S. P. units of vitamin A. It was prepared by adding to the straight oil a concentrate consisting of the non-saponifiable fraction.

TABLE 1  
*Records of calves used in first experiment*

Calf	Breed	Sex	Supplement	Weeks on expt.	Av. daily gain	Muscle pathology
			<i>gram per kilo</i>		<i>lbs.</i>	
3	Holstein	♂	0.1 cod-liver oil	26	2.01	None
7	Holstein	♂	0.1 cod-liver oil	40	2.25	None
10	Guernsey	♂	0.1 cod-liver oil	40	1.78	None
4	Holstein	♀	0.35 cod-liver oil	40	2.34	None
8	Holstein	♂	0.35 cod-liver oil	26	2.25	Slight lesions
5	Holstein	♂	0.7 cod-liver oil	26	2.14	Very slight lesions
6	Holstein	♂	0.7 cod-liver oil	40	2.37	None
9	Guernsey	♀	0.7 cod-liver oil	7		Slight lesions*
1	Holstein	♀	0.1 cod-liver oil (reinforced)	40	1.62	None
2	Holstein	♂	0.1 cod-liver oil (reinforced)	26	1.83	Slight lesions

\* May have resulted from the pneumonia which caused the animal's death.

killed for the same purpose at nine months. The object here was to ascertain whether any injuries evident at six months tended to become more severe with the extension of the feeding period. The growth data presented in Table 1 indicate that all of the animals, with the exception of the one which died, made satisfactory gains for their breed and sex. In fact several made an excellent performance.

Clearly, aside from the case of the one animal which died, no evidence was obtained suggesting that any of the cod-liver levels used interfered with growth or condition. Of course, larger numbers and a check group would have been necessary to reveal any small differences in growth performance.

#### *Post-mortem Examinations*

It was recognized from studies with other herbivora that definite muscle lesions might develop without being sufficiently acute or widespread to cause physical evidence of injury over the periods studied. Thus each animal was subjected to a detailed gross post-mortem examination, and sections were taken from the leg, body and heart muscles, and from the liver, lungs, kidneys, adrenals and thyroid for histological examination. Several sections were taken from each muscle to insure discovery of isolated injuries. The general procedure was the same as previously described (1).

Of the three animals which received the 0.7-gram level of oil, the one which died after 62 days had revealed physical symptoms similar to those of the sheep and goats which had succumbed to the oil injury. The post-mortem examination confirmed the diagnosis of pneumonia, although the

amount of lung congestion seemed insufficient to have caused death. There were; however, no gross symptoms which suggested cod-liver oil injury. On histological examination slight lesions were found in the gastrocnemius muscle but these could not be considered proof of oil injury, in view of the presence of pneumonia which may cause similar lesions according to Forbus (8). Sections from other muscles failed to reveal evidence of dystrophy. The liver contained abundant fat, but it was not of a pathological nature. Thus the examination furnished no certain evidence that the cod-liver oil contributed to the death of this animal.

Calf No. 5 which was killed after receiving the 0.7-gram level of oil for six months appeared entirely normal on gross examination. Histologically, all the leg muscle sections examined showed slight pathological changes, indicative of dystrophy as seen in the other species. The remaining tissues and organs examined were normal. Calf No. 6 which was continued on the highest level of oil for nine months showed no pathological changes either in the gross or histologically.

Calf No. 8 which received the medium level of cod-liver oil was killed and examined at six months of age. The condition noted was almost identical to that observed in the calf on the high level killed at this time. The gross picture was entirely negative as regards pathological changes, but histologically some lesions were present in the muscles of the legs. The injury was too slight to be of any real significance. The other calf on this level of oil, No. 4, was killed at nine months of age. It was normal in all respects according to both the gross and histological examination.

Of the animals which received the 0.1-gram level of oil, neither the one killed at six months nor the two killed at nine months revealed any gross or histological changes on post-mortem examination.

Calf No. 2 which was slaughtered after six months on the reinforced cod-liver oil showed no changes on gross examination, but histologically, isolated fibers of the leg muscles showed slight degeneration. The other animal fed this oil, killed at nine months, appeared entirely normal on post-mortem examination.

The results of this first experiment with calves are in marked contrast with those obtained with sheep and goats (1) where the same oil levels were fed. With these latter species severe lesions resulting in death were noted from the two higher levels whereas in the calves there was no physical evidence of injury, excluding the calf which developed pneumonia, and any histological changes were slight. They were not found at all in the animals continued for the longer period, suggesting the absence of the progressive injury which had been noted in the other herbivora studied.

#### SECOND EXPERIMENT

In view of the results obtained in the first experiment a repetition of the work to obtain data with more animals clearly seemed desirable. In

planning the second trial, it was decided to use a poorer grade of hay, of the kind that would warrant the inclusion of a supplement of vitamins A and D in practice. Accordingly, a timothy hay which was rather stemmy and lacking in color was selected. The choice of this low-protein roughage necessitated a concentrate mixture of higher protein content than the one used in the first experiment. It was made up as follows:

520 pounds gluten feed  
100 pounds cottonseed meal  
200 pounds wheat bran  
60 pounds hominy or corn meal  
220 pounds brewers' dried grains  
140 pounds corn distillers' dried grains  
100 pounds soya bean oil meal  
400 pounds oat feed  
220 pounds molasses  
20 pounds bone meal  
20 pounds salt

A further reason for using this ration was the findings of Turner, Meigs and Converse (9) that with the same intake of cod-liver oil rabbits developed the injury much more quickly and severely on a poor than when on a good ration. With the object of keeping the fat intake low, in view of evidence obtained by Madsen (2) as well as by ourselves that certain fats tended to lessen the injury in guinea pigs, the calves were changed to skim-milk during the first week after birth. Thus the basal ration was chosen with the object of providing the conditions where the cod-liver oil injury would be most likely to develop, based on observations with other species, as well as of providing a ration which in practice would call for the addition of the vitamins supplied by the oil.

Five grade Holstein calves were purchased and brought to the University within three days after birth. Following treatment with anti-scours serum and a few days observation they were placed on experiment in a common pen providing for the individual feeding of the milk. The intake of the latter was progressively increased up to a maximum of 15 pounds per day. At four and one-half months the milk feeding was discontinued. The grain and hay were fed *ad libitum* throughout the experiment. Three of the calves received the 0.7-gram level of the oil and two received the "reinforced" oil at the 0.1-gram level, as shown in Table 2.

During the first four weeks all of the calves grew regularly and appeared thrifty. The growth was somewhat below normal, however, which could be explained as being due to the early change to skim-milk. During the fifth week calf No. 968, receiving the high level of oil, became stiff and showed symptoms of pneumonia. It was killed in a morbid condition ten

TABLE 2  
*Records of calves used in second experiment*

Calf	Sex	Supplement	Weeks on expt.	Av. daily gain	Muscle pathology
		<i>gram per kilo</i>		<i>lbs.</i>	
966	♂	0.7 cod-liver oil	24	1.84	Lesion in one fiber
967	♀	0.7 cod-liver oil	24	1.16	Slight lesions
968	♂	0.7 cod-liver oil	6		None
964	♂	0.1 cod-liver oil (reinforced)	24	1.80	None
965	♂	0.1 cod-liver oil (reinforced)	24	2.27	None

days later, having lost the use of its hind legs. At five weeks another calf, No. 967, developed pneumonia. By careful treatment the trouble was cleared up, although the animal continued to cough until it was three months of age. For a month the calf made little growth, but after three months, growth occurred at a normal rate until it was killed for post-mortem examination at six months of age.

The other three calves remained in good condition throughout the experiment and grew regularly. As is shown in Table 2, the average daily gains for the period as a whole were very good despite a poor growth during the first four weeks. The low average daily gain for No. 967 reflects its set-back caused by pneumonia. At 24 weeks the calves were killed for post-mortem study.

#### *Post-mortem Examinations*

Calf No. 968 which became stiff and died after six weeks revealed a slight pneumonia on post-mortem examination, but not sufficient to cause death. The most marked finding was the presence of a very large, dry mass of hay in the rumen. The physical symptoms before death were very similar to those shown by sheep and goats in which severe muscle lesions were found. Histological examination of this calf failed to show any changes from the normal in the muscles, liver, kidneys or gastrointestinal tract. There was no evidence that the oil was in any way responsible for the failure of the animal.

Calf No. 967 which recovered from pneumonia and grew normally during the last three months showed no pathological changes on gross examination. Histologically, however, both the leg and body muscles showed definite lesions. While these lesions were slight, they were widespread and characteristic of cod-liver oil injury. Since careful study of the lungs showed that the calf had completely recovered from the pneumonia, any muscle lesions caused by this disease should have been healed. Thus it is believed that the lesions found were caused by the oil. Histological studies of the heart, kidneys, liver and thyroids revealed no pathological changes.

The remaining animal receiving the high level of oil was in excellent condition when killed and showed no gross changes on post-mortem. Of the sections taken from the various muscles for histological examination, only one showed any change from normal, and the degeneration was present in only a single fiber. Such an isolated injury is of no significance.

None of the calves which received the "reinforced" oil revealed any evidence of pathological change either in the gross or histologically.

#### DISCUSSION

The results of these two experiments clearly indicate that dairy calves are much less susceptible to cod-liver oil injury than are sheep and goats. With the latter species, according to the previous studies (1) an intake of 0.7 gram per kilo resulted in death or a morbid condition within 90 days, and the 0.35-gram level produced similar results after a longer period. The post-mortem examinations revealed widespread and severe lesions. In contrast, the calves fed these levels showed no physical symptoms of injury over periods of six and nine months. On post-mortem examination most of them were found free from either gross or microscopic lesions and in the others pathological findings were limited to slight microscopic lesions.

The experiments with the different species are not strictly comparable because the rations to which the oil was added were very different. This fact needs emphasis in view of the data previously mentioned indicating that dietary interrelationships have an influence on the degree of injury, but it is not believed that this can be the major explanation of the very different results obtained with the calves than with the other herbivora.

Agduhr and Stenstrom (10) reported that they detected alterations in the heart muscle of calves fed cod-liver oil, by means of the electrocardiogram. In view of this report electrocardiograms were taken at regular intervals on the calves included in the present study. No alterations could be detected as a result of the cod-liver oil feeding. Thus both these studies, which are described elsewhere by Barnes, Davis and McCay (11) and the histological studies here reported failed to confirm the observations of the previous workers as to heart damage in calves from feeding the oil. Here again possible differences due to the nature of the basal rations should be kept in mind.

While the striking feature of the present results is the slight nature of any changes noted, contrary to our previous results with other herbivora and to the results reported by the Swedish workers with calves, our observations do indicate that cod-liver oil can cause muscle injury in calves. At least three of the animals receiving the two higher levels revealed definite, though slight lesions which could not be attributed to any other cause, and one animal receiving the "reinforced" oil showed injury to a similar degree. It is probable that the lesions noted were too slight to interfere with

the normal development of the animals unless further feeding of the oil would have intensified them. Since all the cases of lesions were noted in the animals killed at six months, the animals killed at nine months showing no abnormalities, no evidence was obtained for a progressive injury such as we have frequently noted with the other herbivora studied. Perhaps more marked lesions would have been found if the animals had been examined earlier than at six months.

In the same paper in which their studies with rabbits were described, the Beltsville workers (9) reported observations with calves fed cod-liver oil. An intake of 0.7 gram per kilo caused no evident physical injury, but the high level of two grams per kilo resulted in death. By arrangement with Doctor Meigs tissue sections from some of these calves and from those on later experiments carried out at Beltsville have been studied histologically by us. The findings<sup>2</sup> will be reported in detail from Doctor Meigs' Laboratory. It may be stated, however, that muscle degeneration has been found in ten out of 13 calves studied, ranging from a slight to a moderately severe degree, supporting the observations in our own experiments that cod-liver oil can cause muscle dystrophy in calves. In most cases the intakes fed by the Beltsville workers were higher than those used by us, explaining the greater incidence of injury in their animals.

The observation of cod-liver oil injury in calves has a bearing on feeding practice in that the oil finds some use as a supplement to calf rations to supply vitamins A and D. Long and associates (12) have reported that calves require .3 to .4 U. S. P. units of vitamin D per pound of body weight in addition to the amount supplied by whole milk. Bechdel and associates (13) have reported the much higher requirement of 300 U. S. P. units per 100 pounds live weight where skimmilk was used after four weeks. This intake would be supplied by a U. S. P. grade of cod-liver oil fed at a level of approximately .08 gram per kilo.

Since no sign of injury was noted in the present studies where 0.1 gram per kilo was fed, and since the lesions were slight at higher levels, it appears that the amount of cod-liver oil of U. S. P. grade or higher potency required to furnish the vitamin D needs of the calf can be added to the commonly fed rations used in the present studies without fear of significant harm. Since a much lower intake of a fortified oil would be required, there would seem to be no question about its being a safe source of the vitamins.

The present experiments provide no information as to the conditions under which a supplement of vitamin A or D is needed in a calf ration, and thus the preceding statements should not be interpreted as recommending the general use of cod-liver oil or any other supplement of the vitamins for calves. Evidence such as that obtained by Dahlberg and Maynard (14) indicates that with proper roughage no such supplement is needed.

<sup>2</sup> The writers appreciate the courtesy of Doctor Meigs in allowing them to cite these unpublished results.

## SUMMARY

Dairy calves were reared from birth to six or nine months of age on skimmilk, hay, and grain, supplemented with cod-liver oil at different levels, ranging up to 0.7 gram per kilo. No evidence was obtained that the oil affected adversely the growth or physical condition. On killing the animals for post-mortem study, no gross changes indicative of cod-liver oil injury were found. On histological examination of various muscles, slight dystrophic changes were found in some of the animals, primarily in those fed the higher levels; but the lesions were of a very minor character, in marked contrast to previous findings with other herbivora. The results suggest that cod-liver oil can be fed to dairy calves in the amount that may be required to supply vitamin D without any significant injury.

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# HAY CONSUMPTION OF HOLSTEIN CALVES

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Factors which may influence hay consumption of dairy calves are of interest to many farmers who have a limited amount of grain to feed and who wish to take advantage of the economical growth made by calves when compared with older stock. It is generally recognized that calves do not have a large capacity for feed and that the need of energy for desirable growth necessitates the feeding of more concentrated feeds than hay alone. The questions arise as to what age or weight a calf may consume sufficient roughage to give it the desired energy on hay alone, and what are the factors influencing greater hay consumption. A review of the literature on hay consumption by calves reveals a wide variation in the hay reported as consumed and gives little assistance in answering the questions.

This article reviews some of the work conducted at the Wyoming Agricultural Experiment Station as an attempt to clarify the subject.

## METHOD OF PROCEDURE

The standard for rate of growth was taken from an average of experiment station data as published by Morrison (4). The nutrient intake used as a guide in formulating rations was the Morrison standard as modified by Fitch and Lush (2). The rations fed were designed for farms where whole milk is sold. In formulating rations to conform with the Morrison standard, average analyses of feeds as found in Morrison's "Feeds and Feeding," nineteenth edition, were used.

One group of eight registered Holstein heifer calves, hereafter called Group 1, were fed according to the Morrison feeding standard in individual box stalls from birth to twelve months of age. Whole milk was fed until the calves were about three months of age. For the first three months the grain allowance was unlimited; thereafter, it depended upon the consumption of hay. However, the grain allowance never got below two pounds per head daily. The calves were never allowed to go on pasture, but had access to a dry lot for about five hours during the day. The hay and grain were weighed out to the calves daily, and all refused feeds weighed back. Daily weights of all calves were taken. When the calves were twelve months of age, they were measured for height at the withers, and heart girth.

The grain mixture consisted of three parts ground barley, two parts ground oats, and one part wheat bran. One per cent common salt was added

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to this mixture. Uncut alfalfa hay was fed as a sole roughage. For the most part the alfalfa was of high quality, ranging between 16 per cent and 18 per cent in crude protein. Oat and wheat straw was used for bedding. The calves had access to water in the dry lot and to drinking cups in the barn, except for short periods when the water in the barn had to be shut off to prevent freezing in the pipes.

Another group of eight registered Holstein heifer calves, hereafter known as Group 2, were fed in a manner similar to those in Group 1 until they were about eight months of age. At that time the grain allowance was gradually decreased and the hay allowance increased until the calves were on hay alone at an average of ten months.

### RESULTS

The average hay and grain consumption by weight for Groups 1 and 2 is plotted in Figure 1, which shows that in a single group, when the grain fed

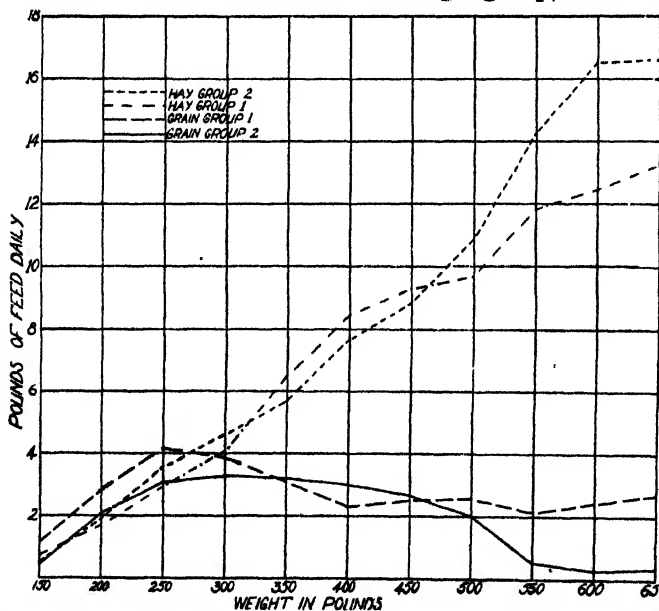


FIG. 1. Comparison of hay and grain consumed according to live weight.

was high, the hay consumption was low. This situation is not necessarily caused by the relative palatability of the hay, although the quality of the hay might have been a factor. When calves in Group 2 had reached about 500 pounds in weight, the hay allowance was increased and the grain decreased, until it was found that the calves were consuming close to the Morrison requirements, using hay alone. The calves were 300 days of age on an average when the grain feeding was discontinued. There are two known factors which made possible this higher consumption of hay: (1) increased allowance of hay, and (2) decreased allowance of grain.

With the grain and hay consumption going in opposite directions it might be anticipated that the calculated total digestible nutrients consumed by the groups would be similar. That such was the case is shown in Figure 2 where

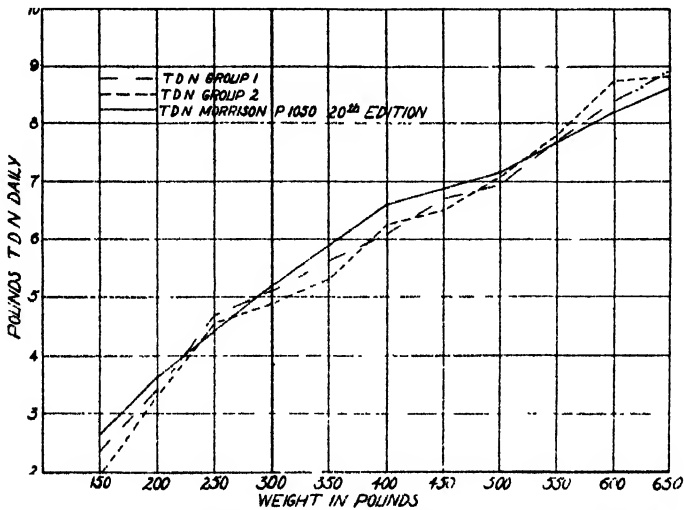


FIG. 2. Calculated and digestible nutrients consumed by Wyoming heifers compared with Morrison's standard.

the T.D.N. consumed by weight is plotted and compared with the average of the Morrison standard.

Increased allowance of hay is a factor influencing hay consumption. Figure 3 shows that the hay consumption of the two groups of calves is related very definitely to hay allowance. It would appear from Figure 3 that Group 1 refused the greater amount of hay. On an average Group 1 refused 12 per cent and Group 2, 7.5 per cent of the hay offered. The greater hay consumption at certain periods of growth in both Groups 1 and 2 was not due to a greater hay allowance alone, but to the amount of grain fed as well, because whenever an increase in the amount of hay fed was made, there was a corresponding decrease in the allowance of grain. To separate the influence of either one of the two factors involves some complications. Considering the feed allowed and consumed between the ages of 180 to 360 days, the calves of Group 1, on an average, were allowed 78 pounds less hay, 143 pounds more grain, and consumed 207 pounds less hay than the calves in Group 2. There was, therefore, a greater difference in consumption between the two groups than there was in hay allowance, indicating that the greater amount of grain fed to calves of Group 1 was a factor depressing the hay consumption. In Figure 4 the grain consumption of each individual calf is plotted from high to low amounts, together with the corresponding hay allowance and consumption, indicating a trend toward a greater consumption of hay with a decreasing grain allowance. However, there are notable exceptions to this

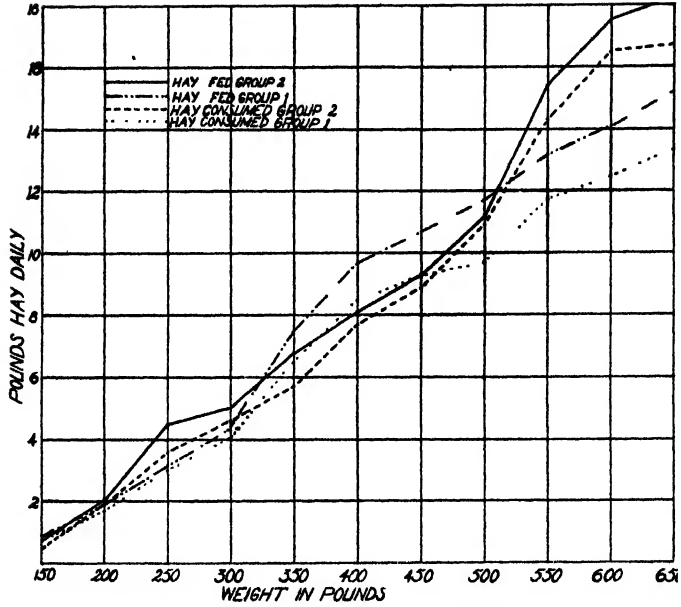


FIG. 3. Comparison of hay allowance with hay consumed.

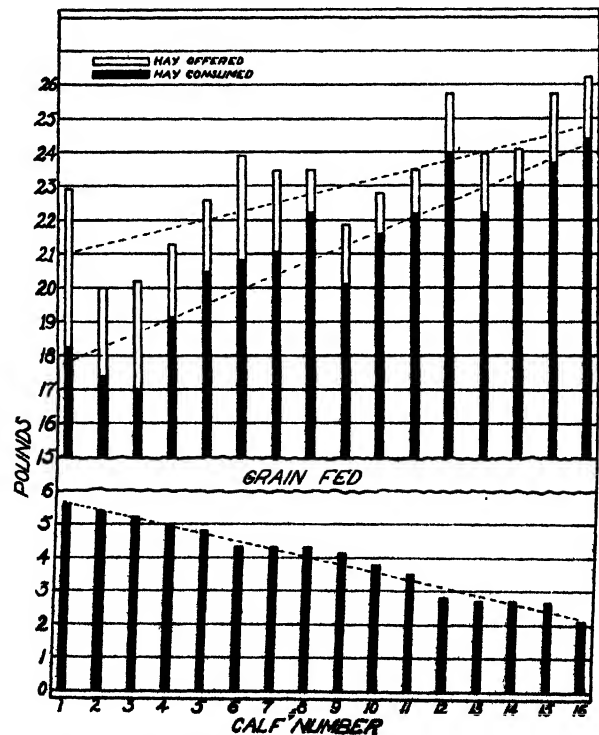


FIG. 4. Effect of grain allowance on hay consumption of 16 calves from 180 to 360 days of age.

trend. Calves 6, 7, and 8 consumed similar amounts of grain, but their hay consumption, indicating a trend toward a greater consumption of hay with Calves 2 and 3, the unusual decline in consumption was due to the smaller hay allowance.

The data in Figure 4 can be used only to indicate trends, since, throughout the experiment, there was a marked difference in the appetites for hay of individual calves.

The rate of growth of the two groups of heifers is compared, in Figure 5,

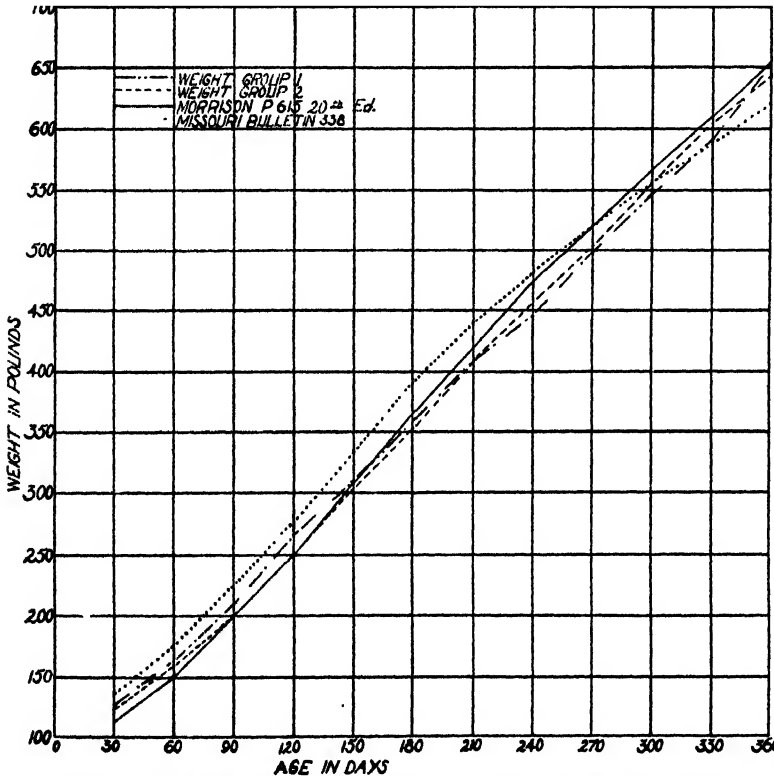


Fig. 5. Weight for age of Wyoming heifers compared with Morrison and Ragsdale.

to that published by Morrison based upon records from several experiment stations, for Holstein heifers (4), and the data by Ragsdale (5). There is, as shown in Figure 5, similar growth curves indicating that the rate of growth of the two Wyoming groups compares favorably with heifers raised where the animals secure greater abundance of feed than they receive on many farms.

#### COMPARISON WITH OTHER DATA

There is little need for comparing the hay consumption of the two groups of Wyoming heifers with that reported in the literature of calf rations, other

than to emphasize possible changes in recommendations in hay allowances when calves are fed on a maximum of high quality alfalfa hay. It was difficult to secure figures comparable with the Wyoming data, because much of the published material gives the total consumption at a given weight or age. In much of the data, skim milk was fed up to six months of age, and silage was fed as part of the roughage from the ages of six to twelve months. For the most part, when consumption of hay is given by weight or age it is recorded as the total or average daily consumption by months. Comparisons with such data, while not exact, at least show trends which are of value.

In Figure 6 the hay consumption of the Wyoming heifers is contrasted

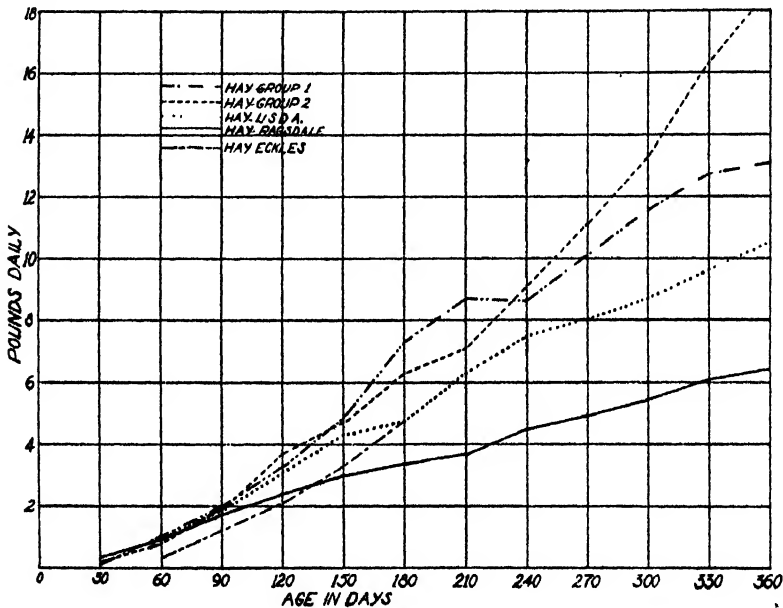


Fig. 6. Hay consumption of Holstein heifers by ages.

with data from three other sources. Eckles (1) reported average daily hay consumption by ten day intervals for Holstein calves from birth to six months of age, fed on rations designed for farms where whole milk is sold. Ragsdale (5) reported the average daily feed consumed by months by Holstein heifers at the Missouri Agricultural Experiment Station, feeding corn silage as part of the roughage. The Shepherd and Miller (6) report on feed consumption also shows that corn silage was fed. In order to compare the roughage consumption in terms of hay, the silage consumption has been divided by 3 and added to the hay consumption, and the total plotted in Figure 6. It is apparent that the roughage consumption reported by Shepherd and Miller comes closest to the Wyoming data, but the spread becomes greater beginning with the eighth month. During the eighth month of age, the average daily hay consumption for Wyoming Group 2 was 12 per cent higher than that

reported by Shepherd and Miller, and the spread continues to widen with increasing age of the heifers. During the twelfth month of age, the Wyoming Group 2 heifers consumed 74 per cent more hay.

#### DISCUSSION

When feeding calves according to a certain total digestible nutrient intake by weight, on rations containing a high percentage of hay, it is difficult to ascertain whether the maximum hay consumption has been reached without a decline in the total digestible nutrient intake of the entire ration. The high hay consumption and similar total digestible nutrient intake was secured in the Wyoming Group 2 heifers simply by trial, lasting sometimes a week or ten days. If, by increasing the hay allowance and decreasing the grain allowance, the heifers failed to consume the required total digestible nutrient intake, then the grain allowance was raised.

Experiments are now under way at the Wyoming Experiment Station to use this method as a means of testing the relative palatability of hay. Exactness in following a schedule for total digestible nutrient intake cannot be expected. In the first place, in experiments of this kind, the exact total digestible nutrient intake would be difficult to secure without a digestion trial. In the second place, heifers vary in weight from day to day, and also vary daily in their hay consumption. Gain in weight and high feed consumption coincide only as a trend and not from day to day.

Using average total digestible nutrient values was satisfactory in these instances, because the object sought was not to find the exact requirements for growth, but rather to find some of the factors which produced great hay consumption so that cheaper growth might be made possible. Net energy values for hay and concentrates may have been more accurate measures here because it is generally recognized that the net energy values of hay and concentrates are farther apart than the total digestible nutrient values would lead one to believe. With all of these possible sources of error in estimating nutrient values, it is interesting to note that the rate of growth of the two groups of calves was practically identical.

#### SUMMARY AND CONCLUSIONS

In this study, two factors have been demonstrated as having an effect toward increasing the consumption of alfalfa hay by dairy calves: (1) an increase of hay allowance, (2) a decrease of grain allowance. This study has further shown that calves may make desirable growth when fed high quality alfalfa hay alone, as early as nine months of age. The experiments also furnish a basis for further study of factors influencing hay consumption.

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# THE USE OF YEAST IN CALF MEALS AND PELLETS<sup>1</sup>

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There has long been a need on dairy farms selling whole milk for a satisfactory method of growing calves with the use of only a small amount of milk.

Remixed dried skimmilk has been used successfully. The increased use of dried skimmilk in human foods has often brought the price of this product up to a point which makes the remixed dried skimmilk method expensive. The labor and care required in remixing the dried milk, heating water, and cleaning pails are also serious disadvantages of this method.

Various gruels have been used. The gruel method, however, is generally unsatisfactory, both from the standpoint of the development of the calves and the labor and care required.

During the past several years progress has been made in raising calves on a small amount of whole milk and various dry calf meals.

A number of studies have been made in which whole milk feeding has been limited to 60 days or less and a simple mixture limited largely to home grains, wheat bran, and linseed meal used (1, 2, 3, 4, 5). The growths of the calves in these studies were quite generally below normal.

Other studies have used dry calf meals containing a wide variety of ingredients and substantial amounts of either dried skimmilk or blood flour or both (5, 6, 7, 8, 9, 10, 11, 12). The results on these various dry calf meals have been somewhat variable. Some have produced very satisfactory growths in calves from the time the calves were weaned from whole milk at from six to ten weeks of age. Others have resulted in growths below normal while the calves were young but by the time the calves were six months of age they were usually of normal size or above.

Previous work at Cornell University (13) on this problem led to the recommendation of a formula for a dry calf starter, and a method of raising calves by the use of this formula.

The purpose of the experimental studies here reported was to test the ability of the recommended method to grow satisfactory calves consistently and to study changes which might further improve it. The latter phase of the work was concerned principally with the use of yeast in the formula and the effects of pelleting the calf starters.

<sup>1</sup> This work was made possible through the financial assistance of Anheuser-Busch, Inc., St. Louis, Missouri. The material included was also presented to the Faculty of the Graduate School of Cornell University, June, 1937, by Paul E. Newman in partial fulfillment of the requirements for the degree of doctor of philosophy.

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Definite information regarding the value of yeast for calves receiving a minimum of whole milk is very limited. Eckles and associates (14) found that the addition of either a dried baker's yeast or a live yeast to the ration of calves receiving whole milk or a combination of whole milk and skimmilk had no effect on the growth or health of the calves. Yeast is known to be high in the vitamin B complex particularly vitamin G (15). Earlier work (16) indicated that what was then (1926) considered vitamin B was not needed in the rations of calves. However, this work was largely with calves after the milk feeding age. In addition the conception of the vitamin B complex has changed greatly since that time.

#### EXPERIMENTAL PROCEDURE

In the studies 65 calves were fed by the dry calf starter method as previously recommended by Cornell University (13). Most of the calves were Holsteins with a few Ayrshires and Guernseys.

The dry calf starter method which was used included the following essential features: Each calf was fed 350 pounds of whole milk. The whole milk fed per day was distributed over the first seven to ten weeks as follows:

POUNDS WHOLE MILK FED DAILY

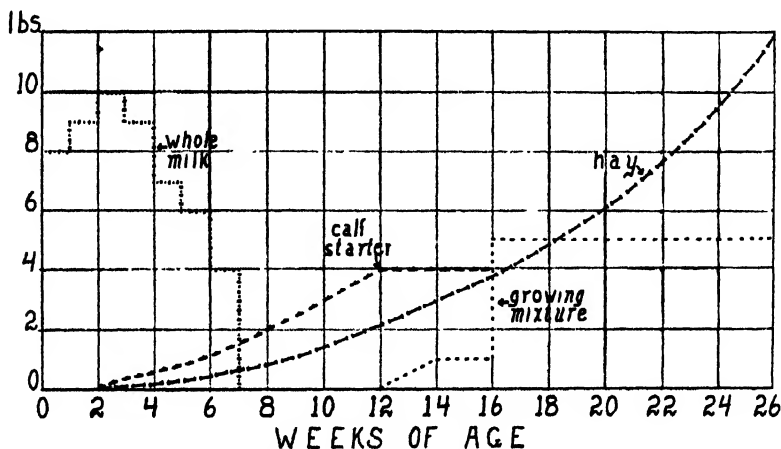
	Holstein	Ayrshire	Guernsey
Calf left with cow first day			
2nd to 7th day, not to exceed	8	7	5
2nd week, not to exceed	9	8	6
3rd " " " "	10	9	7
4th " " " "	9	8	7
5th " " " "	7	7	6
6th " " " "	6	6	6
7th " " " "	4	4	5
8th " " " "	0	3	4
9th " " " "	0	0	3
10th " " " "	0	0	3

Total milk fed was 350 pounds in each case.

An experimental dry calf starter was fed the calves *ad libitum* from two weeks of age until the average daily consumption reached four pounds per calf. It was held at this level until the calf starter was discontinued at sixteen weeks.

After a four pound level of the starter had been reached an additional one pound of a growing mixture was allowed for the remainder of the experimental period in addition to the calf starter. The growing mixture was a 13 per cent protein mixture consisting on a percentage basis of wheat bran 25, corn 25, barley 14, oats 10, linseed meal 10, cane molasses 14, bone meal 1, and salt 1.

Clean fine-stemmed leafy mixed hay was fed the calves *ad libitum* in hay racks beginning at two weeks of age. It was necessary to use three different lots of hay. One was classed as clover medium timothy mixed hay, the second as alfalfa heavy grass mixed hay, and the third as alfalfa heavy timothy mixed hay. A small amount of the second lot graded U. S. No. 1, the balance of the hay used graded U. S. No. 2.



Average daily feed consumption of Holstein calves used in the experiments.

With the exception of the differences in the calf starter formulas which were being studied, all calves were fed and handled as nearly alike as possible. Clean water was available to the calves at all times. Milk was fed the calves individually. The other feeds were fed by groups of two to four experimental calves of approximately the same age.

The experimental period ended at sixteen weeks of age.

The ingredients of the principal experimental calf starter formulas studied are given in Table 1. Formula A, the check formula, is the one previously recommended (13).

The cereal yeast feed used was produced by combining a yeast (Anheuser-Busch strain "L") with corn gluten feed and corn germ meal. This product was approximately 60 per cent corn gluten feed, 20 per cent corn germ meal, and 20 per cent yeast on an 8 per cent moisture basis. It contained 8.0 per cent water, 28.0-29.0 per cent protein, 4.0-4.5 per cent ether extract, 6.0-7.0 per cent fiber, 48-50 per cent nitrogen free extract, and 4.0 per cent ash.

#### RESULTS AND DISCUSSION

Examination of Table 2 shows that on each of the starters the average growth during the experimental period, 2 to 16 weeks of age, was above Ragsdale's normal (17).

TABLE 1  
*Ingredients of experimental calf starters*

Formula	A	AY	GY	GYS	S	3M
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Ground yellow corn	32.25	29.00	26.25	32.25	33.25	26.25
Rolled oats (oat meal)	28.00	25.00	28.00	28.00	28.00	24.00
Wheat bran	10.00	10.00	10.00	10.00	10.00	10.00
Linseed meal	5.00	5.00	5.00	5.00	5.00	5.00
White fish meal	3.00	3.00	3.00	3.00	3.00	3.00
Dried skimmilk	20.00	20.00	10.00	10.00	10.00	30.00
Dried brewers' yeast		6.25				
Cereal yeast feed			16.00	5.00		
Soybean oil meal				5.00	9.00	
Steamed bone meal	.50	.50	.50	.50	.50	.50
Ground limestone	.50	.50	.50	.50	.50	.50
Salt	.50	.50	.50	.50	.50	.50
Cod-liver oil concentrate	.25	.25	.25	.25	.25	.25
Per cent total protein	20.2	22.4	19.4	19.2	20.5	22.1

The average growth of 14 calves on the check formula (A) was 112.8 per cent of normal. For formula AY which had 6.25 per cent dried brewers' yeast added to formula A the average growth of 13 calves was 133.7 per cent of normal. The 9 calves on formula GY in which the dried skimmilk of formula A was reduced to 10 per cent and 16 per cent cereal yeast feed was used averaged 142.3 per cent of normal in growth. In formula GYS the dried skimmilk was reduced to 10 per cent and 5 per cent each of cereal yeast feed and soybean oil meal used. On formula GYS the average growth of 18 calves was 132.9 per cent of normal.

It appears to be quite significant that the growth of the calves on each of the three formulas containing yeast was definitely greater than the growth of the calves on the check formula and approximately one-third greater than what is considered to be normal growth. The numbers are large, 40 calves on the yeast containing rations and 14 on check ration, A.

The general appearance, condition, and body development of the calves on the three yeast formulas was fully as good, and perhaps somewhat better. On all four of the formulas, however, the calves were especially pleasing in these respects.

Formula S in which the dried skimmilk was reduced to 10 per cent and 9 per cent of soybean oil meal but no yeast was used, gave practically as large growths as the check formula (A). The calves were less uniform, however, in growth, appearance, and condition.

The use of 30 per cent dried skimmilk in formula 3M resulted in slower growth than on the check formula. The reason appeared to lie in the lower palatability of the 30 per cent dried skimmilk formula. The bloom and appearance of these calves was very pleasing at all times.

TABLE 2  
*Summary of growths of calves on various formulas*

Formula	Total number of calves	Per cent normal weight		Average daily gain from 2 to 16 weeks, pounds			Per cent normal ave. daily gain 2 to 16 weeks, all breeds	Per cent 16 weeks weight is of 2 weeks weight
		2 weeks	16 weeks	Holstein	Ayrshire	Guernsey		
A . . .	14	91.6	103.4	(11 calves) 1.44	(2 calves) (1 calf) 1.30	(1 calf) .97	112.8 ± 4.0	256.7 ± 5.4
AY . . .	13	98.4	117.9	(10 calves) 1.70	(1 calf) 1.33	(2 calves) 1.34	133.7 ± 5.7	272.4 ± 5.8
GY . . . .	9	102.9	123.1	(5 calves) 1.77	(2 calves) 1.66	(2 calves) 1.28	142.3 ± 5.7	282.5 ± 3.4
GYS . .	18	96.5	116.7	(14 calves) 1.71	(3 calves) 1.60	(1 calf) .85	132.9 ± 4.5	275.4 ± 5.0
S . . . .	6	98.9	109.3	(6 calves) 1.52			117.7 ± 7.1	251.3 ± 6.4
3M . .	5	100.6	101.6	(5 calves) 1.32			102.5 ± 5.9	231.4 ± 13.1

NOTE: In the last two columns of the table are given the means and their standard errors computed according to the following formula:

$$\sqrt{\frac{\sum(X')n - (\sum X)^2}{n^2(n-1)}}$$

The normals used are those of Ragsdale (17).

TABLE 3

*Effects of yeast on feed consumption and efficiency of gains*  
(Only lots consisting entirely of Holsteins are included.)

Formula	A	AY	GY	GYS	S
Number of calves	8	7	4	8	6
Total digestible nutrients consumed per calf, 2 to 16 weeks, pounds	330.6	340.8	360.3	350.3	378.4
Total gain per calf, pounds	138.6	156.6	164.2	157.2	148.9
T.D.N. per 100 pounds gain	238.5	217.6	219.4	222.8	254.1

In Table 3 the relative feed consumption and efficiency of gains on the yeast formulas compared to the check formula and the soybean oil meal formula are shown. The table is only for lots made up entirely of Holsteins as lots of more than one breed could not be included.

Apparently the variation between lots in the consumption of total digestible nutrients was not related to the yeast contents of the formulas. Observations during the experiments indicated that yeast neither increased nor decreased the palatability of the formulas. These figures indicate that in addition to having no influence on palatability yeast had no definite effect on increasing the appetite or total food consumption as is sometimes claimed for it.

The figures on total digestible nutrients required per 100 pounds gain show that the gains were made more efficiently in terms of nutrient utilization on the yeast formulas than on the non-yeast containing formulas.

Half of the calves fed formulas A and AY were fed these formulas in the meal form. The others were fed the same formulas in the pellet form. The pelleting was done by a commercial company equipped to make calf pellets. The pellets were rather hard and approximately three-sixteenths of an inch in diameter.

Table 4 shows that there was no increase in growth, in fact, the growth was somewhat less, on the pellet form compared with the meal form of those two starters. The reason for this is probably due to a lower consumption of the pellets compared with the meal while the calves were young (two to six weeks). This was true for each formula.

TABLE 4

*Average growth of calves fed the meal and pellet forms of formulas A and AY*

Number of calves	Meal	Pellets
	14	13
Average daily gain 2 to 16 weeks, % normal	127.1 ± 4.9	118.3 ± 6.1
% 16 weeks weight is of 2 weeks weight	270.8 ± 6.0	257.2 ± 5.3

## SUMMARY AND CONCLUSIONS

Sixty-five calves were used in studying different calf starter formulas. The following conclusions seem to be warranted:

1. The dry calf starter method in these studies consistently produced calves above normal in weight and of desirable body and skeletal development.
2. The use of dried brewers' yeast and cereal yeast feed in the dry calf starter formulas resulted in greater growth and body development.
3. The total digestible nutrient requirement per unit gain in weight was lower for the yeast-containing calf starters than for the others.
4. The previous level of 20 per cent dried skimmilk can be reduced to 10 per cent without decreasing the effectiveness of the calf starter when yeast or a combination of yeast and soybean oil meal is used to keep the protein level the same.
5. Using a level of 30 per cent dried skimmilk lowered the palatability of the starter and resulted in slower growth.
5. Pelleting of the two formulas studied decreased the consumption of the calf starter by young calves and resulted in slightly slower growth.

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## American Dairy Science Association Announcements

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### THE THIRTY-THIRD ANNUAL MEETING

The thirty-third annual meeting of the American Dairy Science Association will be held at The Ohio State University, Columbus, Ohio, and The Ohio Agricultural Experiment Station, Wooster, Ohio, June 14 to 17, 1938. The concluding sessions on June 17 will be held at Wooster and an interesting general program is being arranged. We are inviting speakers for this part of the program whom everyone in the industry will be interested in hearing.

### LAST CALL FOR TITLES AND ABSTRACTS

The cooperation of each speaker in the prompt submission of titles and abstracts has, in the past, made it possible to have the abstracts published and ready for distribution at the time of the meetings. This year the plan is to have the abstracts of the papers to be presented published and in the hands of the membership before they leave for the meetings. This necessitates the advancement of the deadline for receiving titles and abstracts to a date somewhat earlier than in the past.

April 15 is the deadline, and the program committee will appreciate your cooperation by having your abstract and title at a somewhat earlier date.

Send titles and abstracts to T. S. Sutton, Department of Animal Husbandry, Ohio State University, Columbus, Ohio.

# JOURNAL OF DAIRY SCIENCE

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## BOUND WATER AND ITS RELATION TO SOME DAIRY PRODUCTS

### 1. THE BOUND WATER CONTENT OF SOME MILK PRODUCTS AND OTHER PRODUCTS USED IN THE DAIRY INDUSTRY<sup>1</sup>

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It has been thought for many years that proteins and some other substances owe their stability in milk totally or in part to the degree to which they are hydrated. Some explanations offered to account for many dairy phenomena have been based largely upon this assumption but no quantitative data have been presented to substantiate or refute the theoretical deductions. It is becoming more and more evident that the study of the colloidal state will clarify some of the phenomena that exist in living structures as well as in inanimate colloidal systems like milk and dairy products. In the fields of medicine, biology, physiology, agronomy; biological, physical and industrial chemistry; ceramics, geology, and agriculture, certain phenomena are explained on the basis of hydration of the colloids. It is possible that this colloidal phenomenon may play a part in certain physico-chemical properties of dairy products.

If the fluid dairy product in question lacks colloidal stability, in many cases partial precipitation of the proteins occurs. It is possible that some of the defects that occur in ice cream, milk, evaporated milk, sterilized cream, etc., may be associated with the ability of the milk proteins to bind water or to readsorb water after certain treatments.

Certain substances in the colloidal state have a tendency to hold a quantity of a given liquid with great force. This attraction when exhibited toward water, results in a hydration of the colloidal particles and gives rise to the phenomenon known as water-binding. Water-binding or hydration is usually accompanied with certain physical and chemical changes in the colloidal system. In the case of dairy products it is found that the viscosity may increase, the alcohol stability of the proteins may increase, and a portion of the water in the system will no longer act as a solvent. "Bound Water" in

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<sup>2</sup> The data presented in this paper are from a thesis submitted to the Graduate School of The Pennsylvania State College in partial fulfillment of the degree of Doctor of Philosophy, 1937.

this paper is considered to be that water which will not dissolve sucrose and is the term applied to water held on the surface of colloidal particles, water of hydration, etc. Bound water represents the difference between the total water and the "free" water contained in a system. It is important to recognize that the method used in determining bound water makes an arbitrary division somewhere between water firmly held and that loosely held and that water that is held to such a degree as not to dissolve sucrose is designated as "bound water."

#### HISTORICAL

The specific literature pertaining to the bound water of dairy products is meager. The literature dealing with bound water in other biological systems is much more extensive. In a paper of this nature it would be impractical to cover everything that has been written on bound water as related to other fields. Consequently the literature cited on the bound water of dairy products will be reviewed thoroughly.

Dahlberg (1) tried several methods of establishing the presence of water held physically or chemically in ice cream mixes so that it could not form large ice crystals. The freezing point method which he used is as follows: "Sugar was added to pure water and ice cream mixtures so that the percentage of sugar added when calculated on the water basis was the same in both cases. The sugar dissolved in the same concentration in both cases because the same decreases in freezing points were obtained by the Hortvet cryoscope. This experiment was repeated after the ice cream and water had been set with gelatin in the hope that the gelatin would so retard the diffusion of the sugar that any fixed water would be comparatively free from sugar when the freezing point readings were made. Although differences in the depression of the freezing points were obtained, they were less than  $0.01^{\circ}$  C., and were considered insignificant."

Caulfield (2) made bound water determinations on ice cream mixes using the freezing point technique. The milk solids-not-fat were furnished from condensed skim milk. He reported the percentages of bound water ranging from 8.02 to 8.19 per cent.

Hinkley (3) recalculated Caulfield's data using the correction factor found for the Beckman thermometer used, and the molecular depression constant ( $2.268^{\circ}$  C.) that was obtained. The bound water content was then found to be somewhat lower.

Walts (4), also using the cryoscopic method, determined the bound water content of various dairy products. With a mixture of milk plasma containing 15.17 per cent solids-not-fat he obtained a value of 12.11 per cent bound water after aging at  $40^{\circ}$  F. for 4 hours. He showed that heating milk to pasteurization temperatures and above, lowers the bound water content.

Horvat and Wright (5) determined the bound water content on a mixture containing 65 per cent milk solids, and concluded that, "There is no

evidence of the existence of 'bound' water in solutions of milk solids up to concentrations equivalent to 65 grams solids per 100 grams water."

Mumm (6) studied the hydration of cheese curd using filtration and centrifugal methods and found that when the temperature of determination was varied, a decreased hydration of the casein occurred with increased temperature on acidified and naturally soured milk. In general, increased hydration of the casein occurred on aging. Previously heated milk yielded higher curd volumes than the raw milk. The hydration of albumin was found to decrease with an increase in temperature, but this effect was not as pronounced as in the case of casein. All the salts tried had some effect on the hydration of the casein. Whether an increase or decrease occurred, depends on the pH of the solution.

McDowall and Dolby (7) while working with cheddar cheese noted that a sharp fall in the lactose content of the whey occurs when salt is added to the curd, at whatever stage the salt is added, showing that bound water is liberated. After the curd is cut, lactose is more concentrated in the whey than in the original milk. They calculate that about 2 per cent of the water of milk is bound, *i.e.*, the amount of bound water in cheese curd is about four-fifths (80 per cent) of its casein content. Later McDowall and Dolby (8), with additional evidence, indicate that 0.2-0.3 gram of water per gram of dry matter in the curd is bound.

#### EXPERIMENTAL METHODS

The milk and dairy products used in this investigation were obtained from the College Creamery. The same lot of spray process dry skimmilk was used throughout the investigation.

The "milk plasma mixes" were made from spray process dry skimmilk and distilled water to represent the entire serum solids content of an ice cream mix containing 12 per cent fat, 10 per cent serum solids, 15 per cent sugar, 0.30 per cent gelatin and 62.7 per cent water. These mixes contained then, 10 per cent serum solids from spray process dry skimmilk and 62.7 per cent distilled water, the other ingredients being omitted, because of the difficulty of determining the bound water content in the presence of butterfat, sugar and gelatin. The standard procedure was to pasteurize the mixes at 143° F. for 30 minutes and cool to 40° F. in ice water.

Total solid determinations were obtained in duplicate by the Mojonner method, titratable acidity according to the method of the A. O. A. C. (9), and pH with the quinhydrone electrode.

Protein stability was determined by the use of the alcohol number method. The test is made by noting the least amount of 95 per cent ethyl alcohol necessary to produce an evident feathering in 5 ml. of the sample placed in a test tube and treated first with water and then alcohol, always arranging the additions so that the water and the alcohol equaled 10 ml. The tubes

were inverted several times. Fine particles of curd visible on the inside surface of the test tube indicated the point of destabilization.

The viscosity was measured with a MacMichael viscosimeter, using the disc bob and a No. 27 wire calibrated with standard sucrose solution. These determinations were made in triplicate at exactly 20° C.

The freezing point determinations were made with a Hortvet cryoscope equipped with an improved standardized Beckman thermometer having a 5° C. temperature range graduated to one-hundredth of a degree. The procedure used for the freezing point determinations was as outlined in *The Standard Methods of Milk Analysis* (10) except that super-cooling was usually  $1^{\circ} \pm 0.1^{\circ}$  C.

The sugar used in the bound water determinations was Sucrose C.P. "Pfanstiehl" with a specific rotation of +66.5° and a moisture content of 0.1 per cent. The experimental freezing point depression of 342.24 grams of sucrose dissolved in 1000 grams of pure water was always found to be slightly in excess of that expected for the theoretical depression of 2.085° C. The values obtained, therefore, have been used in this investigation rather than using the theoretical depression or those given by other authors.

The procedure used in making the bound water determinations is in general the same as that described by Newton and Gortner (11). Modifications and a detailed account of the method used are as follows: The freezing point depression of the sample was obtained by the use of the Hortvet cryoscope. Corrections for under-cooling were made by the use of the table given by Harris (12).

The total solid content having been previously obtained by the Mojonnier method, a portion of the sample being investigated, containing exactly 100 grams of water, was weighed out. This was a convenient quantity to permit the determination of the freezing point in duplicate or triplicate, if necessary. The same portion of the sample was never used twice. The sample must be weighed accurately as the principle of the method depends upon the determination of small differences in the amount of free water present in the sample. To the weighed portion of the sample were added 34.224 grams of pulverized C.P. Sucrose, just sufficient to make a molar concentration in the total water present. (It was later found that adding the sample to the weighed beaker and sugar gave better results.) The sucrose samples were previously weighed into small glass beakers and stored in a desiccator over sulfuric acid until required. Twenty minutes of frequent stirring with a glass rod was used before the freezing point of the solution was determined. The sucrose had been ground in a Wiley mill so that it would dissolve more readily.

The freezing point depression was again obtained on the solution containing the sucrose and the correction for under-cooling again applied. The

depression was usually found to be greater than the sum of the values for the sample and the sugar solution when determined separately.

It is assumed from these results that not all of the calculated 100 grams of water in the sample is in the free state, a certain quantity being held as "bound" water by colloids present. The amount of water available for dissolving the sucrose is equal to 100 grams less the quantity "bound." The molar concentration of sugar is, therefore, increased more than the theoretical amount, and this will be shown by an excess depression of the freezing point. The magnitude of the excess depression is taken as a measure of the water held in such a way as not to be available for the solution of sucrose which is designated in this paper as bound water.

The formula of Newton and Gortner (11) used to calculate the per cent of bound water is as follows:

$$\text{Per cent Bound Water} = \frac{\Delta_x \times 89.2}{\Delta_s}$$

Where:

$\Delta$  = the freezing point depression of the sample.

$\Delta_a$  = the freezing point depression of the sample after adding 34.224 grams of sucrose (1/10 mole.) to a quantity of sample containing 100 grams of water.

$\Delta_s = \Delta_a - \Delta$  (the actual depression of the freezing point due to sucrose).

$\Delta_x = \Delta_s - 2.148$ ,<sup>\*</sup> the amount (determined experimentally) by which the depression, found on addition of the sucrose is in excess of that expected over the theoretical depression.

89.2 = one mole. hexahydrate dissolved in 1000 - (18 × 6) or 892 grams of water. Since 100 grams of water are used the factor becomes 89.2 in the one-tenth molar solution of sucrose.

### *The Bound Water Content of Milk and Some Milk Products*

Some earlier work by Caulfield (2), Hinkley (3) and Walts (4) indicated that milk and milk products are hydrophilic in nature and that the cryoscopic method could be used to measure the bound water content of liquid dairy products.

To determine if milk and milk products contain appreciable amounts of bound water, several samples were obtained and the bound water content determined. The amount of water bound depends on the total solids content and such factors as salt balance, acidity, and protein stability, either natural or acquired through some previous treatment. Consequently, two samples having the same solids content may vary in bound water content. Bound water content was determined on milk, skimmilk, cream, buttermilk, condensed skimmilk, and colostrum milk. These data together with other studies on viscosity, and protein stability, are given in Table 1.

<sup>\*</sup> In other lots of sucrose this figure becomes 2.132, 2.165 and 2.160 respectively.

TABLE 1

*The bound water content of some milk and milk products*

Sample	Hours aged at 40° F.	Per cent solids	pH	Vis- cosity centi- poises	Per cent acid	Alco- hol num- ber	Per cent bound water
1. Raw Milk	24	13.25	6.49	2.387	0.205	8.2	3.18
2. Skimmilk	24	9.44	6.51	1.877	0.210	8.5	2.13
3. Cream	8	29.08	6.66	8.928	0.145	..	2.50
4. Cream	8	43.20	6.73	31.683	0.135	..	3.42
5. Buttermilk	0	8.25	5.70	1.797	0.290	3.0	1.75
6. Cond. Skim- milk	24	25.33	6.15	7.650	0.580	5.4	11.62
7. Colostrum	24	19.17	6.12	11.120	0.330	5.2	4.65

Throughout this investigation it was found that normal whole milk binds approximately 2 to 3.5 per cent water. Skimmilk binds less water than whole milk, the reason being, as will be later shown, that the fat globule "membrane" is hydrophilic. Cream has associated with it some bound water and it will be noticed that as the fat content increases the bound water content also increases. Sweet cream buttermilk, on the average, binds slightly less water than does skimmilk. Condensed skimmilk, since it is concentrated, binds relatively a large percentage of water.

#### *Water Bound by Some of the Constituents of Milk*

The substances in milk thought to have hydrophilic properties are casein, lactalbumin, globulin, and colloidal di-calcium phosphate. It has been found in this work that the fat globule "membrane" also binds a portion of the water in milk. This study, therefore, was undertaken to determine what substances in milk bind water and to what extent this water is bound by these substances.

A fresh sample of whole mixed milk from the college herd was used to determine the relative amounts of water bound by each of the constituents. The milk was divided into five lots as follows: (1) fresh whole milk; (2) skimmilk obtained by separating the whole milk; (3) skimmilk treated with rennet to coagulate the casein and to obtain the serum, and the pH regulated with sodium bicarbonate; (4) same as (3) except that the serum was brought to a pH 4.7 with acetic acid; and (5) serum at a pH 4.7 boiled to precipitate the albumin and then filtered. This milk contained 12.10 per cent total solids, 8.85 per cent plasma solids, 3.25 per cent fat, 2.34 per cent casein, 0.65 per cent albumin, and 5.86 per cent solids remaining including lactose, salts and soluble proteins. From the data obtained it was possible to calculate the amount of water bound by each of the above constituents in the concentrations as they appear in milk. The results of this experiment are given in Table 2.

TABLE 2  
*The water bound by the constituents of milk (rennet coagulation)*

Sample	Hours aged at 40° F.	Per cent solids	pH	Viscosity centipoises	Per cent acid	Alcohol No.	Per cent bound water	Grams bound water per gram solids
1. Whole Milk	4	12.10	6.56	2.352	0.165	8.3	2.88	0.238
	24	12.10	6.56	2.382	0.170	8.4	2.92	0.241
2. Skimmilk	4	8.85	6.57	2.088	0.175	8.4	2.58	0.291
	24	8.85	6.57	2.088	0.180	8.5	2.58	0.291
3. Serum at pH 6.58	4	6.51	6.51	1.794	0.105		0.89	0.136
	24	6.51	6.52	1.794	0.105		0.97	0.149
4. Serum at pH 4.76	4	6.40	4.76	1.705	0.245		0.44	0.068
	24	6.40	4.75	1.705	0.250		0.44	0.068
5. Remaining Solids	4	5.86	6.23	1.617	0.095		0.12	0.020
	24	5.86	6.25	1.617	0.100		0.12	0.020

The data in Table 2 show that bound water decreases when the colloidal material is removed from the milk. That is, the data show that on the removal of the hydrophilic colloids by precipitation and filtration the excess depression of the freezing point becomes less and less until it becomes practically nil in sample No. 5. This, consequently, gives proof that emulsoid colloids do in fact bind part of the water.

From these results it was found that milk containing 2.92 per cent bound water after 24 hours aging at 40° F. had a distribution of its bound water content as recorded in Table 3.

TABLE 3

*Water bound by the constituents of milk expressed on gram and percentage basis (rennet coagulation)*

Constituent	Per cent in milk	Grams bound water per gram material	Percentage of the water bound
1. Casein .....	2.34	0.688	55.14
2. Albumin .....	0.65	1.30	29.11
3. Fat + Butterfat Membrane	3.25	0.104	11.65
4. Remaining Solids	5.86	0.020	4.10

It may be seen that casein binds about 55 per cent of the water that is bound in milk, albumin approximately 29 per cent and the fat plus the "membrane" in the concentration found in milk 11.65 per cent, while the remaining solids which consist of lactose, minerals and possibly a small amount of colloidal material binds 4.10 per cent of the bound water.

On a gram basis it is noticed that the albumin is more hydrophilic than the casein and the fat plus the "membrane" is less hydrophilic than either the casein or the albumin. Nevertheless, it will be shown later that the fat globule "membrane" when isolated from the fat globules binds approximately the same amount as the casein.

The data in Table 2 also show that at a pH of 6.52 the serum binds approximately twice as much water as it does at a pH of 4.75. This shows that as the isoelectric zone is reached less water is bound by the albumin, but precipitation does not occur unless further dehydration by alcohol or heat is practiced.

Another experiment was conducted to determine if precipitation with acetic acid gave any different results than precipitation with rennet on the water bound by the constituents of milk. The pH was also adjusted where necessary with sodium bicarbonate. The data obtained are given in Table 4.

The data in Table 4 when analyzed show practically the same results as outlined previously with rennet coagulation. Although the milk was higher in total solids content the amount of water bound by this sample was practically the same as in the previous experiment.

TABLE 4  
*The water bound by the constituents of milk (acid coagulation)*

Sample	Hours aged at 40° F.	Per cent solids	pH	Viscosity centipoises	Per cent acid	Alcohol No.	Per cent bound water	Grams bound water per gram solids
1. Whole Milk	4	13.72	6.59	2,382	0.195	8.6	2.60	0.189
	24	13.72	6.58	2,441	0.195	8.7	2.91	0.212
2. Skimmilk	4	9.12	6.60	2,058	0.205	8.7	2.29	0.251
	24	9.12	6.60	2,117	0.205	8.7	2.37	0.259
3. Serum at pH 6.5	4	6.72	6.58	1,798	0.18	..	0.90	0.133
	24	6.72	6.58	1,798	0.18	..	0.94	0.139
4. Serum at pH 4.7	4	6.64	4.76	1,718	0.46	..	0.39	0.058
	24	6.64	4.76	1,718	0.46	..	0.39	0.058
5. Remaining Solids	4	6.07	6.39	1,624	0.17	..	0.08	0.013
	24	6.07	6.40	1,624	0.17	..	0.08	0.013

The analysis of the milk together with the amount of water bound on a gram and percentage basis is given in Table 5.

TABLE 5  
*Water bound by the constituents of milk expressed on gram and percentage basis (acid coagulation)*

Constituent	Per cent in milk	Grams bound water per gram material	Percentage of the water bound
1. Casein .....	2.40	0.596	49.10
2. Albumin .....	0.65	1.32	29.55
3. Fat + Butterfat Membrane	4.60	0.117	18.55
4. Remaining Solids .....	6.07	0.013	2.74

From the results of analysis this milk contained 13.72 per cent of total solids, 9.12 per cent plasma solids, and 4.60 per cent fat.

A comparison with the results in Table 3 show that with the greater percentage of fat there is more water bound since there is more "membrane" material present.

The results of these experiments show that casein binds approximately 50 per cent of the water that is bound in milk. The casein content of normal cow's milk varies between 2.25 per cent and 2.75 per cent and since it is present in greater quantities than any of the other colloid substances, it probably plays the greatest role in the colloidal properties of milk.

#### *The Bound Water of Whey Protein and Lactose Solutions*

In Tables 3 and 5 it was shown that the albumin present in milk is about twice as hydrophilic as casein. Undoubtedly it also plays a role in the colloidal properties of dairy products but since it is more of a true hydrophilic colloid than casein, factors that may affect casein may not affect albumin.

Experiments were conducted with some whey protein powder obtained from the Bureau of Dairy Industry, U. S. D. A. This powder had been electrodialed and contained 6.51 per cent ash, 1.22 per cent moisture, 22.71 per cent whey protein, and 69.56 per cent lactose. At first it was thought desirable to determine the bound water of the whey protein powder in the concentration found in milk at pH values of approximately 4.8 and 6.7 and compare the results with some of the data recorded in Tables 2 and 4 with the milk serum.

Another sample of whey protein powder was dialyzed in collodion membrane bags until the tests for chlorides and lactose were negative. The distilled water was changed at least once a day. The samples were kept at a temperature of 38° F. while dialyzing and toluene was used as a preservative. The object was to obtain the whey proteins as free of salts and lactose as possible and to determine the bound water content on the purified whey proteins. To note whether lactose affected the bound water determinations, a

lactose solution was prepared and the bound water determined in the usual manner. The results of these studies are given in Table 6.

TABLE 6  
*The bound water content of whey protein, dialyzed whey protein,  
and lactose solutions*

Sample	Hours aged at 40° F.	Per cent solids	pH	Vis- cosity centi- poises	Per cent acid	Per cent bound water	Grams bound water per gram solids
1. Whey Protein Powder* pH 4.82 ..	4 24	5.99 4.99	4.81 4.82	1.291 1.352	0.27 0.27	0.66 0.74	0.110 0.123
2. Whey Protein Powder* pH 6.67 ..	4 24	6.53 6.53	6.66 6.67	1.411 1.470	0.125 0.13	2.17 2.37	0.333 0.362
3. Whey Protein Powder* pH 6.78 .....	4 24	5.67 5.67	6.79 6.78	1.441 1.500	0.135 0.14	2.07 2.23	0.365 0.393
4. Pure Whey Protein	1 wk.	0.28	7.58	1.147	0.00	0.21	0.750
5. Pure Whey Protein	4 da.	0.57	6.55	1.294	0.01	0.38	0.650
6. Lactose	4 24	4.42 4.42	5.73 5.73	1.088 1.088	0.00 0.00	0.12 0.12	0.027 0.027

\* This sample contains lactose and minerals of milk.

The results obtained with whey protein sols, made from a special whey powder, showed that these whey proteins bound considerably more water than the whey proteins found naturally in milk (Tables 2 and 4). There is more water bound by the whey proteins at a pH of 6.67 than at a pH of 4.82 and this would seem to indicate that at the isoelectric point there is less water bound by the whey proteins than at the normal pH of milk.

The whey powder proteins did not bind as much water as the whey protein obtained by dialyzing in a collodion bag until free from electrolytes. Possibly the explanation lies in the fact that natural sols bind more water than prepared sols as has been reported by Newton and Martin (13).

The bound water studies with lactose indicate that it does affect the bound water determinations of dairy products. The slight amount of water bound can be attributed to the experimental error of the method employed.

#### *The Bound Water Content of Cream of Various Fat Percentages*

During the course of the work on cream, it was noticed that creams of various fat percentages bound approximately the same amount of water per gram of total solids. To obtain more information, creams of approximately 18 per cent, 31 per cent, and 40 per cent fat were produced and bound water determinations made after aging 4 and 24 hours at 40° F. (Table 7).

TABLE 7  
*The bound water content of cream of various fat percentages*

Per cent fat in cream	Hours aged	Per cent solids	pH	Vis- cosity centi- poises	Per cent acid	Per cent bound water	Grams bound water per gram solids
1. 18.08 .....	4	25.79	6.59	5.735	0.14	4.71	0.182
	24	25.79	6.57	6.029	0.145	4.82	0.186
2. 30.93 ... ..	4	37.25	6.65	21.176	0.12	6.88	0.187
	24	37.25	6.63	22.352	0.125	7.08	0.190
3. 40.33 .....	4	45.68	6.72	31.176	0.105	8.59	0.188
	24	45.68	6.74	33.235	0.105	8.93	0.195

Although the 18 per cent cream contained approximately 7.71 per cent of solids-not-fat and the 40 per cent cream 5.35 per cent solids-not-fat, the bound water content per gram of total solids remained apparently the same. The explanation for this phenomenon lies in the fact that the fat globule membrane is hydrophilic to about the same degree as the casein. Later results show that this hypothesis is correct.

Perlman (14) shows that the phospholipid content of cream increases uniformly with the fat content up to approximately 55–58 per cent, after which it diminishes with further increases in fat content. He also shows that the phospholipid content per unit of fat remains fairly uniform in cream containing between 20 per cent and 60 per cent fat. It can be concluded from the data that on an equal solid basis creams of various fat percentages in the range studied will bind approximately the same amount of water per gram of solids.

*The Bound Water Content of the Fat Globule Membrane  
and Pure Milk and Egg Phospholipids*

In this study the fat globule "membrane" was obtained according to the method of Palmer and Wiese (15). The isolation of pure milk and egg phospholipids was based on the procedure outlined by Bull (16) and noted by Josephson and Dahle (17).

The pure milk phospholipids, even though waxy and fat-like in appearance, disperse uniformly in water to form a cloudy viscous sol. Bound water determinations were made on sols of the fat globule "membrane," pure milk and egg phospholipids. The results obtained are recorded in Table 8.

From the results obtained in Table 8, pure milk phospholipids appear to be more hydrophilic than any substance studied. A sol of the milk phospholipids containing 0.63 per cent solids bound 6.46 grams of water per gram of solids after 24 hours aging. It is apparent that the bound water content of the fat globule membrane is almost a linear function of concentration. It will be remembered similar results were obtained with cream (Table 7) where

TABLE 8

*The bound water content of fat globule membrane and pure milk and egg phospholipids*

Sample	Hours aged at 40° F.	Per cent solids	pH	Viscosity centipoises	Per cent acid	Per cent bound water	Grams bound water per gram solids
1. Fat Globule Membrane	4	2.83	6.54	1.470	0.04	1.63	0.58
2. Conc. Fat Globule Membrane	24	2.83	6.54	1.614	0.04	1.79	0.63
3. Pure Milk Phospholipids	4	10.60	6.91	6.117	0.095	5.65	0.53
4. Pure Egg Phospholipids	24	10.60	6.90	6.264	0.10	6.33	0.59
	4	0.63	6.76	2.294	0.01	3.88	6.16
	24	0.63	6.74	2.353	0.01	4.05	6.46
	4	1.08	6.23	2.353	0.01	1.93	1.79
	24	1.08	6.23	2.382	0.01	2.05	1.90

the bound water content was attributed to the hydrophilic properties of the fat globule membrane.

Pure egg phospholipids bound relatively less water per gram solids than pure milk phospholipids. A sol containing 1.08 per cent of total solids showed after 24 hours aging 1.90 grams of bound water for each gram of solids. It is possible that some of the original properties of the egg phospholipids may have been altered as the material was prepared from dried egg yolk. There is also a marked difference in certain other properties of the phospholipids obtained from different sources as Jack (18) found the isoelectric point of milk phospholipids to be pH 2.0, while Price (19) found the isoelectric point of egg lecithin to be pH 2.7.

On an equal solids basis it seems that the viscosity of the milk phospholipids is greater than that of the egg phospholipids. From the bound water results obtained this would seem to be correct as the milk phospholipids are more hydrated. The pH of milk phospholipids was slightly lower than that obtained with egg phospholipids.

*The Bound Water Content of Some Gums and Stabilizers  
Used in the Dairy Industry*

In the past, and also to some extent at present, gums have been used as stabilizers in ice cream, ices and sherbets, because they have the property of absorbing water and the amount absorbed may be many times their weight. Some of these gums and stabilizers form gels at higher concentration, while others remain as sols. Their action in ice cream, ices and sherbets is to produce a finer texture by preventing formation of large ice crystals in the frozen product.

Studies were made on gum arabic, oat flour, egg yolk, locust bean flour, "colace," gelatin and sodium alginate sols.

Gum arabic, although not used extensively in dairy products, was used to study the effect of concentration on the bound water content since it does

not gel upon increasing the concentration. Approximately 1.4 and 8 per cent sols were made and compared.

Another experiment was conducted with oat flour (Avenex No. 7) to determine whether this material is hydrophilic and also to determine the effect of concentration on the bound water content. Oat flour has anti-oxygenic properties and when used in ice cream also acts as a stabilizer. Oat flour mixtures were made so that water extract solutions of approximately 0.5, 1.0 and 2.0 per cent resulted.

The bound water content of egg yolk, locust bean flour and "colace" was determined on approximately one per cent sols.

Approximately 0.5 per cent gelatin sols were prepared from 175, 200 and 250 Bloom gelatins to study the hydrophilic properties of this stabilizer. Three different concentrations of sodium alginate were also studied and these results together with those of gum arabic, oat flour, egg yolk, locust bean gum, and "colace" are given in Table 9.

TABLE 9

*The bound water content of some gums and stabilizers used in the dairy industry*

Sample	Hours aged at 40° F.	Per cent solids	pH	Viscosity centipoises	Per cent acid	Per cent bound water	Grams bound water per gram solids
1. Gum Arabic . . .	4	0.93	4.90	2.054	.. ..	0.37	0.40
	24	0.93	4.86	2.054	.. ..	0.37	0.40
2. Gum Arabic . . . .	4	4.32	4.54	4.701	.. ..	2.36	0.55
	24	4.32	4.54	4.701	.. ..	2.39	0.55
3. Gum Arabic . . .	4	8.15	4.39	8.533	.. ..	4.81	0.59
	24	8.15	4.39	8.236	.. ..	4.72	0.58
4. Oat Flour 'Ex-tract	4	0.49	6.29	1.041	0.01	0.12	0.241
	24	0.49	6.31	1.041	0.01	0.16	0.326
5. Oat Flour Ex-tract	4	1.23	6.32	1.143	0.015	0.24	0.195
	24	1.23	6.36	1.143	0.015	0.32	0.260
6. Oat Flour Ex-tract	4	2.00	6.34	1.388	0.02	0.45	0.225
	24	2.00	6.36	1.388	0.02	0.49	0.245
7. Dried Egg Yolk ..	4	1.18	5.51	1.294	0.03	0.89	0.75
	24	1.18	5.53	1.323	0.03	1.09	0.92
8. Locust Bean Flour	4	1.05	5.06	132.352	0.005	1.40	1.33
	24	1.05	5.04	135.294	0.005	2.03	1.93
9. "Colace" .....	4	1.22	4.55	100.588	0.005	0.86	0.70
	24	1.22	4.56	102.941	0.005	1.12	0.91
10. Gelatin 175 Bloom	4	0.50	4.24	1.481	.. ..	- 0.31	.....
	24	0.50	4.26	2.212	.....	- 0.20	.....
11. Gelatin 200 Bloom	4	0.57	4.41	2.054	.. ..	- 0.56	.....
	24	0.57	4.39	3.081	.....	- 0.31	.....
12. Gelatin 250 Bloom	4	0.57	4.44	3.239	.....	- 0.52	.....
	24	0.57	4.54	11.535	.....	- 0.07	.....
13. Sodium Alginate	4	0.59	8.64	9.411	.....	- 1.61	.....
	24	0.59	8.64	9.411	.....	- 1.61	.....
14. Sodium Alginate	4	1.36	9.11	39.411	.....	0.33	0.242
	24	1.36	9.11	39.705	.....	0.33	0.242
15. Sodium Alginate	4	2.53	9.30	119.117	.....	0.65	0.257
	24	2.53	9.30	120.000	.....	0.65	0.257

With most colloids the bound water per gram of colloid tends to diminish with the concentration of the sols. Gum arabic seems to be an exception. Over the range studied bound water appears to be almost a linear function of concentration. This characteristic does not hold for all gum arabic. Newton and Gortner (11) found, in their original experiments on which the cryoscopic method of determining bound water was based, that there is a logarithmic relation between bound water content and concentration in this gum arabic. Later Newton and Martin (13) obtained results to show that bound water is almost a linear function of concentration with some gum arabic.

Samples numbers 4, 5 and 6 show that the water extract of oat flour is not very hydrophilic and binds a relatively small amount of water per gram of extractable material. Nevertheless, the relative insolubility of oat flour should not prevent it from binding water. The non-colloidal fraction, therefore, may be responsible for the stabilizing properties of oat flour. Newton and Martin (13) show that although gelatin, agar, and blood fibrin are relatively difficult to disperse in cold water, they bound more water than substances colloiddally dissolved.

The results show that egg yolk, locust bean flour and "colace," which contains locust bean flour, are quite hydrophilic in nature and some of the properties they impart to ice cream are probably due to this factor. The viscosities of the sols of locust bean flour and "colace" are very great which is typical of most gums and stabilizers.

From the results obtained with gelatin and sodium alginate in Table 9, it is apparent that the cryoscopic method cannot be used to determine the bound water content of substances of this nature, since the water held by these substances will dissolve sucrose. In fact, in most instances, negative amounts of bound water were obtained. It is possible that the structure of these sols is different from other sols, or a slight positive adsorption of the sucrose would account for these findings; that no water was "bound."

Sayre (20) points out that, "If water held by gelatin and agar is considered as bound water, then this method fails to give a real measure of bound water, since much of the water held by those substances will dissolve sucrose." Nevertheless, Versmold (21), Newton and Martin (13) and others report the presence of bound water in agar and gelatin when the cryoscopic technique is used.

These data bring out the fact that gelatin increases in viscosity on aging while sodium alginate does not and this correlates with the fact that ice cream mixes made with sodium alginate also do not increase noticeably in viscosity on aging, while gelatin mixes usually do.

#### SUMMARY AND CONCLUSIONS

The cryoscopic method can be readily used for the determination of bound water in liquid dairy products with few exceptions.

Milk, cream and other liquid dairy products contain appreciable amounts of bound water. In a milk of average fat and total solids content, the casein binds approximately 50 per cent of the bound water, the albumin 30 per cent, the fat globule "membrane" 15 per cent, and the remaining solids less than 4 per cent.

There is some indication that rennet casein binds more water than acid casein and therefore this fact may contribute to some of the differences of the two caseins.

More water is bound by the true hydrophilic colloids (whey proteins) of milk at a pH 6.6 than at a pH 4.7. Dialyzed whey protein bind as much water as natural whey proteins found in milk. Lactose does not affect bound water determinations of liquid dairy products with the cryoscopic method.

A large portion of the bound water present in cream is due to the hydrophilic properties of the fat globule "membrane" and in cream containing between 18 and 40 per cent butterfat the bound water content per gram of solids remains fairly constant.

The fat globule membrane binds approximately the same amount of water as the casein. Pure milk phospholipids are found to be the most hydrophilic of any substances isolated from milk in this study. Pure egg phospholipids are less hydrophilic than the pure milk phospholipids.

In this study the bound water content of gum arabic appears to be almost a linear function of concentration. The colloidal extract of oat flour (Avenex No. 7) is not very hydrophilic. Powdered egg yolk, locust bean flour and "colace" bind relatively large quantities of water while gelatin and sodium alginate give negative results, in some instances, with the cryoscopic method.

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# BACTERIAL CONTENT AND KEEPING QUALITY OF BUTTER AFTER REMOVAL FROM STORAGE

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Recent practices in butter storage plants have tended toward lower holding temperatures and in some cases temperatures considerably below freezing have been employed. The present investigation was outlined to study the influence of freezing on the types and numbers of bacteria in butter and also to follow the subsequent changes in flavor and bacterial content when the butter was removed from frozen storage. The practical value of holding butter below freezing depends not only on the keeping quality protection during holding but also on the influence of such holding on the keeping quality of the frozen butter after it has reached the distributor, retailer or consumer.

## REVIEW OF LITERATURE

Only a few reports from the extensive literature on the influence of low temperature storage on butter will be included here. A more inclusive review has been published by the author (2) in a previous paper.

In 1906, Gray and McKay (3) proved that butter held at  $-10^{\circ}$  F. kept better both while in cold storage and after removal from storage than when stored at higher temperatures. Rogers and coworkers (4) obtained similar results and indicated that the difference between the results at  $-10^{\circ}$  F. and  $0^{\circ}$  F. was enough to warrant the use of the lower temperature.

The changes in the bacterial content of butter stored below freezing have been studied by a number of investigators. In many cases the results have shown no significant changes, although the tendency has been for decreases in counts when temperatures much below the freezing point were used. Washburn and Dahlberg (5) concluded that bacteria in unsalted butter decreased more rapidly at  $-15^{\circ}$  F. than they did in salted butter, but little if any relationship was noted between the numbers of bacteria and the butter score after storage. Grimes (6) held salted butter from ripened and un-ripened cream for a period of six months at  $-6^{\circ}$  F. and found that marked decreases in counts occurred, the greatest decrease resulting in the ripened cream butter. Grimes and Hennerty (7) studied the changes in numbers of microorganisms in sweet cream, salted butter held for periods of from two to eight months at  $15^{\circ}$  F. The results showed no significant change in bac-

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terial counts, an increase in yeast and a definite decrease in mold counts. No definite relationship could be noted between the changes in numbers of bacteria, yeasts or molds and the change in butter score during storage.

Since they found no significant changes in yeast, mold, or bacterial counts in butter held at  $-7^{\circ}\text{C}$ . ( $19^{\circ}\text{F}$ .) for six months, Arup and Gilmore (8) concluded that the flavor deterioration which resulted was of chemical origin. Loftus-Hills, Scharp and Bellair (9) arrived at similar conclusions on salted butter stored at  $12^{\circ}\text{F}$ .

#### METHODS OF PROCEDURE

The trials include fifteen churnings from which both salted and unsalted butter were obtained. The portions of salted and unsalted butter were each divided into two portions and placed in five-ounce glass jars, one of which was placed at  $-25^{\circ}\text{C}$ . and the other held at room temperature ( $21^{\circ}\text{C}$ .). Plate counts were made on the fresh butter, on the samples held at  $-25^{\circ}\text{C}$ . after one day and 90 days, and on the samples held at room temperature after 2, 4 and 7 days.

After the butter had been held for 90 days at  $-25^{\circ}\text{C}$ . it was held for an additional 7 days at room temperature and bacterial counts and flavor examinations were made after 2, 4 and 7 days. The results on this butter were used for comparison with the results obtained on the corresponding butter held 7 days at room temperature when fresh.

The portions for analysis were obtained from the surface and subsurface of the samples by means of a sterile spatula. The numbers of total, lipolytic and proteolytic bacteria were determined by the methods recommended by the committee on Bacteriological Methods (1). The total bacterial counts were made on beef infusion agar while the lipolytic and proteolytic bacteria were determined on a special plating using the beef infusion agar with oil emulsion, Nile blue sulphate solution and sterile skimmilk added.

Flavor examinations were made on the butter when fresh, and after holding under the various conditions described above. The butter was not scored but was described as satisfactory or defective in flavor and comments were recorded on the types of flavors detected.

The "room temperature" designation is used in the tables and discussion to indicate a temperature within  $\pm 1^{\circ}$  of  $21^{\circ}\text{C}$ .

#### RESULTS AND DISCUSSION

##### *Changes in Numbers of Bacteria in Butter Held at $-25^{\circ}\text{C}$ .*

The results given in Table 1 indicate that freezing effected a significant reduction in the numbers of bacteria in both salted and unsalted butter. The reduction in numbers during holding was much greater in the unsalted butter than in the salted butter even though the counts in the salted butter were

TABLE 1

*Influence of holding at -25° C. on the bacterial counts of salted and unsalted butter*

Held at -25° C.	Numbers of bacteria per ml., geometric means of 15 lots of	
	Unsalted butter	Salted butter
0 days . . . . .	877,000	161,000
1 day . . . . .	167,000	101,000
90 days . . . . .	65,000	29,400

lower at the end of the holding period. These reports agree in general with those of Washburn and Dahlberg (5) who found more rapid decreases in counts in unsalted than in salted butter held at -15° F. (-26° C.)

The marked difference between the bacterial counts on the salted and unsalted butter when fresh was due undoubtedly to the destructive action of salt. The comparatively small decrease in the numbers in the salted butter after holding at -25° C., however, indicated that the salt effected some protective influence at the low temperature and prevented the destruction of as large a percentage of the bacteria as were destroyed in the unsalted butter. The freezing point lowering due to the salt brine in the butter serum appears as the best explanation of the protective action to the bacterial flora in the salted butter. The presence of salt may have prevented crushing to some extent by interfering with the complete crystallization of the moisture in the butter. The salt brine concentration in the butter was approximately 16 per cent and a brine of this concentration has a freezing point of -12.2 C.

Lipolytic and proteolytic bacteria were not detected on the plates poured from the butter after 1 day at -25° C. even though dilutions as low as 1:100 were regularly made. These results indicate that the small numbers of lipolytic and proteolytic bacteria present in the freshly made butter were inactivated or destroyed in the freezing process.

#### *Bacterial Counts in Fresh and Storage Butter Held at Room Temperature*

Table 2 presents a summary of the changes in numbers of bacteria in butter held at room temperature after storage at -25° C. for 90 days and in the corresponding butter held at room temperature when fresh. The numbers of bacteria increased more rapidly in the unsalted storage butter than in the corresponding fresh unsalted butter when held at room temperature. The bacterial counts on the two types of butter were approximately the same after the 7 days, even though the initial counts on the storage butter were comparatively lower. The numbers of bacteria, decreased in both the fresh and storage salted butter when held at room temperature and were somewhat lower on the storage butter after 7 days.

TABLE 2

*Changes in bacterial counts on fresh butter and on storage butter held 7 days at room temperature*

Butter	Number of churnings	Number of bacteria per ml., geometric means of 15 lots of			
		Unsalted butter		Salted butter	
		0 days	7 days	0 days	7 days
Fresh .....	15	877,000	22,500,000	161,000	50,900
Storage .....	15	65,000	20,300,000	29,400	22,900

*Flavor Deterioration in Fresh and Storage Unsalted Butter Held at Room Temperature*

A comparison of the flavor deterioration in 15 lots of fresh and 15 lots of storage, unsalted butter held at room temperature is presented in Table 3. Since no off-flavors were noted previous to the 4-day examination, the table gives the data for only the 4- and 7-day examinations. A flavor defect developed in only one lot of the fresh butter during the first 4 days, while six lots of the storage butter showed some flavor deterioration at 4 days. After 7 days there was no significant difference in the number of lots of fresh and of storage butter showing flavor defects, but the flavor defects were more pronounced in the storage butter than they were in the fresh butter. Flavor defects were detected after 7 days in certain lots of fresh butter when they were not detected in the corresponding storage lots, and vice versa. The

TABLE 3

*Flavor deterioration in fresh and in storage unsalted butter held 7 days at room temperature*

Churning No.	Flavor comments			
	After 4 days holding		After 7 days holding	
	Fresh butter	Storage butter	Fresh butter	Storage butter
1 .....	---	---	---	---
2 .....	---	sl. off	---	Roquefort
3 .....	---	sl. off	sl. off	sl. off
4 .....	sl. rancid	oily	rancid	sl. bitter
5 .....	---	---	rancid	---
6 .....	---	sl. off	sl. off	sl. off
7 .....	---	---	sour	---
8 .....	---	---	sl. off	---
9 .....	---	---	---	---
10 .....	---	---	---	Roquefort
11 .....	---	---	sl. off	Roquefort
12 .....	---	sl. oily	sl. rancid	Roquefort
13 .....	---	---	---	Roquefort
14 .....	---	sl. oily	sl. oily	moldy
15 .....	---	---	---	sl. oily

--- = flavor satisfactory; sl. = slightly.

TABLE 4

*Lipolytic and proteolytic bacteria in unsalted butter held 7 days at room temperature after removal from storage at -25° C.*

(Time flavor deterioration first noted—indicated by \*)

Churning No.	Type of bacteria	Numbers of bacteria per ml.			Flavor defects
		2 days	4 days	7 days	7 days
1	Lip. Prot.	<10,000 <10,000	<10,000 <10,000	<10,000 <10,000	
2	Lip. Prot.	<10,000 630,000	<10,000* 3,000,000	<10,000 34,000,000	Roquefort
3	Lip. Prot.	130,000 <10,000	45,000* 500,000	10,000 15,000	slightly off
4	Lip. Prot.	300,000 500,000	200,000* 100,000	400,000 1,000,000	slightly bitter
5	Lip. Prot.	20,000 <10,000	85,000 <10,000	<10,000 <10,000	
6	Lip. Prot.	xxx xxx	4,300,000* 100,000	4,700,000 <10,000	slightly off
7	Lip. Prot.	390,000 15,000	1,400,000 1,200,000	500,000 850,000	
8	Lip. Prot.	355,000 100,000	150,000 135,000	55,000 25,000	
9	Lip. Prot.	5,600,000 900,000	4,900,000 1,900,000	1,550,000 1,900,000	
10	Lip. Prot.	4,500 5,000	20,000 55,000	5,000* 10,000	Roquefort
11	Lip. Prot.	160,000 200,000	450,000 6,200,000	8,000,000* 16,000,000	Roquefort
12	Lip. Prot.	130,000 50,000	2,200,000* 1,600,000	6,000,000 7,500,000	Roquefort
13	Lip. Prot.	55,000 45,000	30,000 32,000	160,000* 170,000	Roquefort
14	Lip. Prot.	7,500 6,500	<1,000* 5,000	<1,000 5,000	moldy
15	Lip. Prot.	35,000 35,000	20,000 190,000	2,100,000* 2,100,000	slightly oily

xxx too many to count with dilutions used.

most common defects noted were rancid and Roquefort flavors; there were two lots of fresh butter and five lots of storage butter developing one or the other of these defects. It is evident from these results that the storage butter failed to keep as well when placed at room temperature as the corresponding lots of fresh butter.

Flavor defects other than tallowiness were not developed in either the fresh or storage, salted butter during the 7 days at room temperature and consequently no comparison of keeping quality from a bacteriological standpoint could be made.

*Lipolytic and Proteolytic Bacteria in Unsalted Butter Held 7 Days at Room Temperature After Removal from Storage at  $-25^{\circ}$  C.*

The changes in the numbers of lipolytic and proteolytic bacteria in the 15 lots of unsalted storage butter held at room temperature are shown in Table 4. Although lipolytic and proteolytic bacteria were not detected in the platings of fresh butter there were increasingly large numbers evident in some of the lots after holding at room temperature. In the ten lots which showed flavor deterioration at 7 days, the numbers of lipolytic bacteria ranged from less than 1,000 to 8,000,000 per ml. The five lots, which kept, contained from less than 10,000 to 1,550,000 lipolytic bacteria per ml. The numbers of proteolytic bacteria in the butter which developed flavor defects ranged from 5,000 to 34,000,000 per ml. while the lots which kept ranged from less than 10,000 to 1,900,000 per ml. No correlation between the numbers of lipolytic or proteolytic bacteria and specific flavor defects could be noted. The lots which developed rancid flavor did not contain larger numbers of lipolytic bacteria than some of the lots which showed either indefinite flavor defects or no flavor defects. A similar lack of agreement existed between the numbers of proteolytic bacteria and flavor defects. Neither lipolytic nor proteolytic bacteria were detected in the salted butter held 7 days at room temperature subsequent to storage.

SUMMARY

Holding at  $-25^{\circ}$  C. effected marked decreases in the total numbers of bacteria present in both salted and unsalted butter but the decreases were much less pronounced in the salted butter than in the corresponding unsalted butter. The destructive action of salt was apparently of greater importance than freezing in reducing the numbers of bacteria in salted butter. No change in flavor was detected in the butter which had been frozen for 90 days.

The bacterial counts increased more rapidly and flavor deterioration was more rapid in the unsalted butter held at room temperature ( $21^{\circ}$  C.) after storage at  $-25^{\circ}$  C. than in the fresh butter held 7 days at room temperature.

Large numbers of lipolytic and proteolytic bacteria were found in certain lots of unsalted butter after holding at room temperature but no definite correlation could be noted between the growth of these types of bacteria and the development of flavor defects.

The bacterial counts did not change significantly in salted butter held 7 days at room temperature subsequent to storage at  $-25^{\circ}$  C. Neither lipolytic

nor proteolytic bacteria were detected in the salted butter which had been held 7 days at room temperature subsequent to storage at  $-25^{\circ}\text{C}$ . Flavor defects other than tallowiness were not detected in the salted butter when it was held 7 days at room temperature subsequent to storage at  $-25^{\circ}\text{C}$ .

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# A METHOD FOR THE EXTRACTION OF FAT IN ICE CREAM IN ORDER TO DETERMINE ITS PURITY<sup>1</sup>

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In our ice cream studies the last three years it has been necessary to extract large quantities of fat from the same sample of ice cream. At different times we have used various suggestive methods. After numerous trials, we confined our work to a modified Mojonner method, using a large separatory funnel. In spite of its apparent simplicity, it frequently happened that undue emulsification occurred with the use of ammonium hydroxide causing, thereby, a loss of time and increasing the amount of reagents used.

Since the official methods (A. O. A. C. Fourth Edition, 1935) (1) does not contain a method for the extraction of fat applicable to ice cream, we have, therefore, attempted to devise a method for the rapid extraction of the fat in larger than test quantities. In order to overcome the tendency toward emulsification, various acids were tried in the place of the base, ammonium hydroxide. It was found that glacial acetic acid produced a more rapid uniform separation of the fat and there was no tendency toward charring as there was with sulphuric acid.

## PROCEDURE

Two hundred grams of ice cream mix or melted ice cream was washed into a 500 ml. separatory funnel with about 50 ml. of warm distilled water. To this was added 30 ml. of glacial acetic acid and the contents shaken occasionally over a period of 15-20 minutes after which 100 ml. ethyl alcohol was added and again shaken until homogeneous. The stopper was loosened frequently to avoid pressure in the funnel. Then 75 ml. each of ethyl and petroleum ethers was added in order mentioned. The mixture was shaken thoroughly after each addition. This mixture was allowed to stand until the ether-fat layer had completely separated. The lower layer, *i.e.*, ice cream mix, was then drawn off into another separatory funnel, extracted again using 50 ml. of each of the two ethers. (One hundred ml. of the mixture of the recovered ethers may be used for the second extraction.) After the fat-ether layer was completely separated, the lower layer was discarded and the two-ether layers were combined in one separatory funnel. These were washed three times with distilled water, using about 150 ml. each time. The fat-

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TABLE 1  
*Comparison of methods for the extraction of fat from ice cream*

Method	Chocolate		Vanilla		Extracted Fat % Yield		Remarks
	R. M. No.	K. Sap. No.	R. M. No.	K. Sap. No.	1st	2nd	
Acetic acid	24.53	224.2	28.45	227.8	75-80	5-8	Clear fat
Ammonium hydroxide	24.64	225.7	28.58	229.3	25-40	2-3	Clear fat
Sulphuric acid	24.74	225.3	28.71	228.9	70-75	5-7	Charred or darkened fat

ether mixture was then transferred to a 500 ml. Erlenmeyer flask and the ethers were distilled off of the fat. When most of the ethers were distilled off, the fat was filtered into a sample container using a short stem funnel. The filtering of the fat can best be done in an electric oven at 60° to 75° C. if the above distillation has been previously carried out. The filtered fat was then heated to about 95–98° C. for one hour to free it from possible traces of volatile substances.

Ice cream containing fruits should first be filtered through a gauze strainer to remove the fruit particles before washing into the separatory funnel.

#### RESULTS

Table 1 shows the comparison of the Reichert-Meissl and saponification numbers, expected yield and color of fat extracted from vanilla and chocolate ice creams. The results in Table 1 are the average of four samples of each type of ice cream picked at random from several samples. The expected yield of fat from the acetic acid method is about 75 per cent for the first extraction and about 5 to 8 per cent in the second extraction. The emulsification of the fat was quite evident in the method where ammonium hydroxide was used as shown by the low yield of fat. It is also probable that some saponification of the fat occurred as noted by the persistent lather produced. The yield of fat with the ammonium method varied considerably with different samples. The sulphuric acid method gave a much darker fat than the other methods, probably due to the fact that the sugar in the ice cream was charred and carried over with the ether-fat mixture. The Reichert-Meissl and saponification numbers of the fat in chocolate ice creams are lower than pure butterfat since it contains cocoa fat from the chocolate or cocoa (2). It will be noted from the table that the fat constants for the three methods checked very closely.

#### SUMMARY

A method is given for the extraction of fat from ice cream in large quantities.

Results obtained in the extraction of fat from ice cream indicate that the acetic acid method is more economical, more fat is secured per extraction and takes less time than either the sulphuric acid or ammonium hydroxide methods.

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# THE CHEMICAL COMPOSITION AND PROPERTIES OF NORMAL AND RANCID JERSEY MILK

## I. CHLORIDE AND LACTOSE CONTENT

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During a study of methods of milk scoring, certain off-flavors were frequently observed in the milk of the college Jersey herd. Numerous inquiries as to the cause of undesirable flavors in milk emphasized the economic significance of the problem. A systematic study was made, therefore, of the occurrence of off-flavors in milk, for the purpose of determining the cause and means of prevention of their development.

Natural rancidity proved to be the most pronounced off-flavor encountered in the study. Approximately ten per cent of 928 samples scored for flavor were criticized as rancid. Frequently the flavor was so offensive that it made the milk unpalatable. "Rancid" milk, designated by some investigators as the "bitter milk of late lactation," was first described in detail by Eckles and Shaw (5). Koestler *et al.* (11) have described such milk as having a peculiar biting sharp after-taste which changes to a definite sharp acid-taste resembling rancid butter when allowed to stand at room temperature. The frequent occurrence of this flavor is indicated by Roadhouse and Henderson (14) in a survey of abnormal flavors occurring in milk from twelve commercial herds. Of 536 animals observed, 68 produced milk with abnormal tastes or flavors and of these, 17 gave naturally rancid milk.

Despite the interest which has been shown in the causes of the production of rancid milk, no systematic study appears to have been made of the occurrence of rancidity in the milk of the animals of a given herd, nor any comparison made of the chemical composition of rancid milk with that of normal milk produced under the same conditions. The present paper presents the chloride and lactose content of normal and rancid milk produced by Jersey cows maintained under regulated conditions. Subsequent papers deal with the fat, total solids, protein, lipase, titratable acidity and hydrogen-ion content of normal and rancid Jersey milk.

It is commonly recognized that the breed of animal and the period of lactation affect the composition of milk, yet there is a marked scarcity of data sufficiently detailed to serve as criteria of normal values for the different constituents at all stages of lactation. Allen (1), in a review of the mineral constituents and citric acid content of milk, discussed the lack of detailed data showing the influence of the period of lactation upon the mineral content of milk. He stated that although it is a well-known phe-

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nomenon that milk undergoes marked changes in composition as the period of lactation advances, the number of workers who have actually given detailed data for the analysis of milk at definite lactation periods is surprisingly small. In discussing the effect of the period of lactation upon the chloride content of milk, he refers to four investigations (8, 10, 16, 17), none of which were sufficiently detailed to be considered conclusive. Recent work has been reported by Sharp and Struble (15) and Caulfield and Riddell (3).

Lactose was considered by early workers (12, 5) as one of the least variable constituents of milk, but later work (6) showed that the lactose content of the milk of individuals may vary considerably from the average of the herd. Eckles and Shaw (7) found that the lactose of the milk of an individual animal may vary 0.5 per cent from the average lactose content, but that 90 per cent of the samples obtained during a given lactation show a variation of less than 0.2 per cent.

#### EXPERIMENTAL

The cows of the college Jersey herd were selected for observation. The management of the animals did not differ from the practice usual with the college herds. The animals were kept out-of-doors both day and night during the entire experiment except on stormy nights during the winter months. The same ration was fed throughout the experiment except that the cows were on pasture in the summer. On the days when milk samples were collected the cows were always kept off pasture and were allowed no hay or silage for five hours previous to milking. This precaution was necessary to avoid development of feed flavors. In addition to pasture in season, roughage was supplied by alfalfa, prairie hay and darso silage. The concentrate mixture used contained by weight 1 part of cottonseed meal, 4 parts of ground corn, and 2 parts each of ground oats and wheat bran and was fed in proportion to the milk yield.

The cows were milked at 12-hour intervals and individual samples representative of an entire milking were obtained once a week from each animal in the herd. Samples were taken at the evening milking since preliminary work showed no appreciable differences in the flavor of the morning and evening milk. They were kept in a refrigerator over night and the following morning were scored for flavor by the method previously reported by Fouts and Weaver (9).

Determinations of the chloride and lactose content of the individual samples were made over a period of 2 years. During this time, observations were made on 20 animals, not all of which were continuously available for the entire period. Of the 20 cows observed, 13 were from 2 to 4 years old, 4 were from 5 to 6 years, and 3 were from 11 to 12 years. At no time during the study were any of the animals reported by the station veterinarian to be suffering from mastitis, either in an acute or chronic form.

## ANALYSIS OF SAMPLES

The chloride content of the milk was determined by the method of Van Slyke and Donleavy (18) as modified by Dennis and Sisson (4). In this method, 20 cc. of a 1.2 per cent solution of picric acid and 20 cc. of standard silver nitrate are added to 10 cc. of milk. Precipitation of the protein is instantaneous and complete, and after standing 10 minutes the liquid is poured on a chlorine-free filter. An aliquot of the clear filtrate is taken and the excess of silver is titrated with potassium iodide solution.

In the analysis for lactose, the protein of milk was precipitated with copper sulphate and sodium hydroxide as described in the Methods of Analysis of the A. O. A. C. Precipitation of cuprous oxide was carried out by the official method of Munson and Walker. The estimation of cuprous oxide was made by the volumetric method described by Bisson and Sewell (2).

## PRESENTATION OF DATA

*Chloride Content of Normal Jersey Milk*

Graphs in Figure 1 show the chloride content of milk samples obtained weekly during a complete lactation period of each of ten animals. Two lactations are shown for animal 7. The milk of animals 1 through 7 (lactation 1) may be regarded as typical of normal milk of the herd.

Data used in Figure 1 are presented statistically in Table 1. The standard deviations and coefficients of variation indicate a wide variation in the mean chloride content of the milk of an animal during a given lactation. Variation between individuals is shown by a range of from 0.091 per cent to 0.136 per cent in the mean chloride content of the milk of the ten animals.

The average chloride content of normal milk of the herd for each week of lactation is shown in Figure 2. This curve was established by the analysis of 690 samples. The general trend of the chloride during lactation is characterized by an initially high value which falls during the first four weeks, then rises gradually until about the 38th week when the rate of increase becomes more pronounced. This rapid rise continues until the end of lactation.

Statistical constants for the mean chloride content of normal milk of the herd are given in Table 2 for 12 four-week periods. No values are included for samples criticized either as "salty" or "rancid." The standard errors set down in Table 2 show the reliability which may be attributed to the calculated means. The degree of dispersion of the chloride of the milk of different animals in the same period of lactation is high and is approximately the same as the variation in the chloride of the milk of the individual animals during lactation.

*Chloride Content of Rancid Milk*

The production of rancid samples by the animals shown in Figure 1 is typical of the herd. Some of the cows produced no rancid milk; others gave

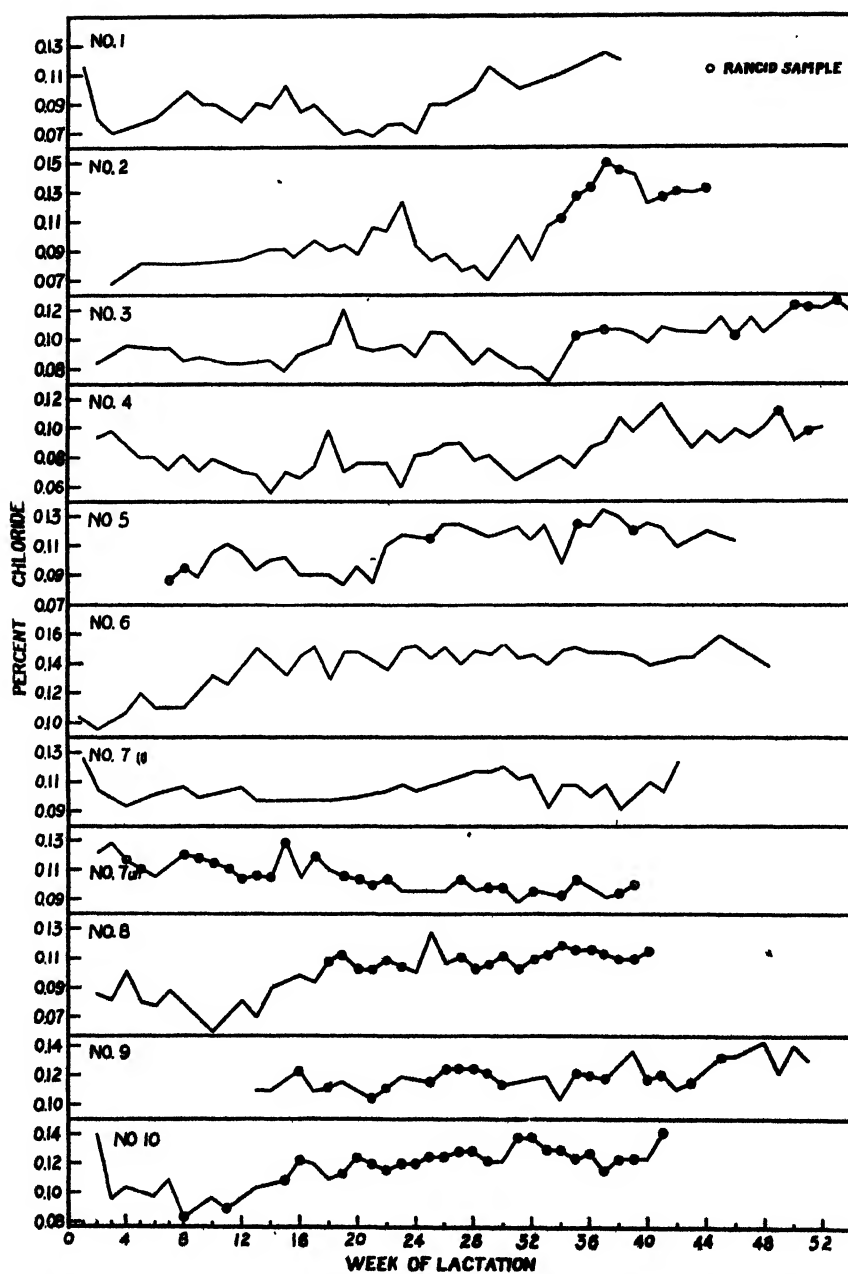


FIG. 1. Chloride content of milk samples taken weekly from each of ten Jersey cows during a complete lactation. Rancid samples are indicated by circles.

TABLE 1  
Chloride and lactose content of milk produced by ten<sup>1</sup> Jersey cows during a complete lactation

Cow number	Age years	Milk samples	Chloride content			Milk samples	Lactose content		
			Mean chloride	Standard deviation	Coefficients of variation		Mean lactose	Standard deviation	Coefficients of variation
		number	per cent	per cent	per cent	number	per cent	per cent	per cent
1	10	37	0.091 ± 0.003 <sup>2</sup>	0.016	17.3	36	4.97 ± 0.04 <sup>5</sup>	0.27	5.4
2	4	38	0.099 ± 0.004	0.022	22.1	41	4.78 ± 0.05	0.32	6.6
3	4	52	0.100 ± 0.002	0.014	14.0	44	4.89 ± 0.04	0.23	4.7
4	3	67	0.089 ± 0.002	0.015	16.3	66	4.93 ± 0.04	0.29	5.9
5	4	40	0.114 ± 0.003	0.022	18.9	38	4.89 ± 0.05	0.29	4.6
6	12	48 <sup>3</sup>	0.136 ± 0.002	0.016	12.0	45 <sup>3</sup>	4.28 ± 0.03	0.18	4.1
7 (1)	11	36	0.105 ± 0.002	0.011	9.9	36	4.66 ± 0.06	0.37	7.8
7 (2)	12	36 <sup>4</sup>	0.107 ± 0.002	0.010	9.4	29 <sup>4</sup>	4.93 ± 0.04	0.23	7.8
8	3	39 <sup>4</sup>	0.101 ± 0.002	0.012	11.7	34 <sup>4</sup>	4.73 ± 0.05	0.29	6.1
9	12	43 <sup>4</sup>	0.127 ± 0.002	0.015	11.7	39 <sup>4</sup>	4.12 ± 0.04	0.27	6.7
10	7	39 <sup>4</sup>	0.117 ± 0.002	0.015	12.7	39 <sup>4</sup>	4.67 ± 0.05	0.28	6.0

<sup>1</sup> Two lactations are shown for cow number 7.

<sup>2</sup> Milk criticized as salty during entire lactation.

<sup>3</sup> Chloride decreased, lactose increased with advancing lactation.

<sup>4</sup> More than 50 per cent of the samples were criticized as "rancid."

<sup>5</sup> Standard error of mean.

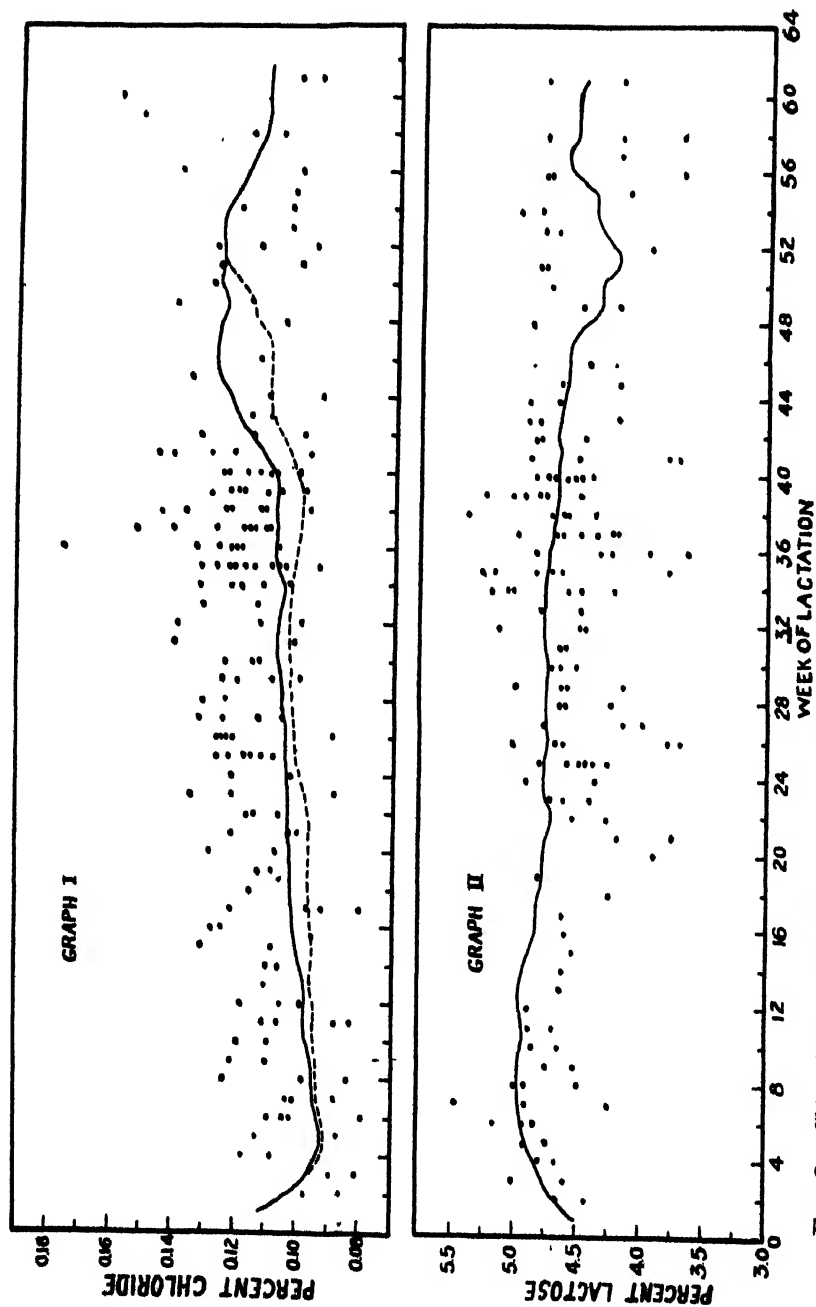


FIG. 2. Chloride and lactose content of Jersey milk.

Graph I ——— Mean chloride content of all normal and salty samples for each week of the lactation period.  
 - - - - - Mean chloride content of all normal samples.  
 • Chloride content of rancid samples.

Graph II ——— Mean lactose content of all normal samples for each week of the lactation period.  
 • Lactose content of rancid samples.

TABLE 2  
Chloride and lactose content of normal<sup>1</sup> Jersey milk in relation to the period of lactation

Lactation period	Milk samples	Chloride content			Milk samples	Lactose content		
		Mean chloride	Standard deviation	Coefficients of variation		Mean lactose	Standard deviation	Coefficients of variation
weeks	number	per cent	per cent	per cent	number	per cent	per cent	per cent
1-4	65	0.097 ± 0.002 <sup>2</sup>	0.016	16.7	63	4.75 ± 0.043 <sup>2</sup>	0.34	7.3
5-8	67	0.094 ± 0.002	0.013	13.6	63	4.93 ± 0.032	0.25	5.1
9-12	50	0.095 ± 0.002	0.011	12.1	49	4.94 ± 0.048	0.34	6.8
13-16	61	0.095 ± 0.002	0.012	13.0	58	4.85 ± 0.051	0.39	8.1
17-20	43	0.097 ± 0.002	0.014	9.7	45	4.80 ± 0.070	0.47	9.8
21-24	50	0.097 ± 0.002	0.014	14.6	50	4.74 ± 0.048	0.34	7.1
25-28	59	0.100 ± 0.002	0.015	14.9	62	4.81 ± 0.047	0.37	7.7
29-32	62	0.102 ± 0.003	0.021	20.7	65	4.78 ± 0.058	0.46	9.7
33-36	47	0.103 ± 0.003	0.018	17.1	52	4.78 ± 0.059	0.43	8.9
37-40	29	0.103 ± 0.004	0.020	19.0	36	4.69 ± 0.062	0.37	7.9
41-44	23	0.108 ± 0.003	0.014	12.6	30	4.62 ± 0.068	0.37	8.1
45-48	13	0.108 ± 0.003	0.010	9.4	20	4.61 ± 0.042	0.42	9.2

<sup>1</sup> Samples criticized as "salty" or "rancid" are not included.

<sup>2</sup> Standard error of mean.

a few such samples, usually toward the end of lactation; while others produced rancid milk as early as the 4th and 8th weeks of lactation and continued to produce it throughout the remainder of the lactation period.

As may be seen from Figure 1 and Table 1, the chloride of the milk of animals most frequently producing rancid samples was usually higher for a greater part of the lactation period than it was for animals which produced few or no rancid samples. For example, the milk of animals 8, 9 and 10, frequently rancid, showed a consistently high chloride content after the 16th week of lactation, the values ranging from 0.110 per cent to 0.140 per cent. However, during the weeks in which these cows produced normal milk the chloride showed no change. That the high chloride may be characteristic of all the milk of the animal and not of the rancid samples alone is also indicated in the two lactation periods of animal 7. The chloride content of the milk was high in both lactations, although no rancidity occurred until the second lactation.

In order to facilitate a comparison of the chloride content of rancid milk with that of normal milk produced at the same stage of lactation, the chloride content of 179 rancid samples is shown as individual points in Figure 2, in conjunction with the curve of the average chloride content of normal milk. It is evident from the figure that the chloride of the rancid samples was, in most instances, higher than the average chloride of normal milk produced at the same stage of lactation.

If the period of lactation is disregarded, a further comparison of the chloride of normal and rancid milk may be made by grouping the samples as shown in Table 3. The mean chloride content of all rancid samples was  $0.1139 \pm 0.0012$  per cent, that of the normal samples, exclusive of salty samples,  $0.0983 \pm 0.0008$  per cent. The difference between the means of the two groups, 0.0157 per cent, is 11 times the standard error of the difference, 0.0014, and is, therefore, statistically significant.

In considering the chloride of milk, it is of interest to observe the chloride content of samples which were judged "salty." Of 530 samples which had a chloride content of from 0.050 per cent to 0.120 per cent, 25 were criticized as salty, 15 of the "salty" samples containing from 0.101 per cent to 0.120 per cent chloride; of 82 samples containing 0.121 per cent to 0.140 per cent chloride, 34 were considered salty; while in 48 out of 51 samples containing more than 0.141 per cent chloride the salty taste was perceptible.

#### *Lactose Content of Normal Jersey Milk*

The graphs in Figure 3 show the changes occurring in the lactose content of the milk of individual animals with the advance of lactation. Variation between different animals in the lactose content of the milk may also be observed for all stages of the lactation period. The lactose content of the milk of animals 1 through 7 (lactation 1) may be regarded as typical for the

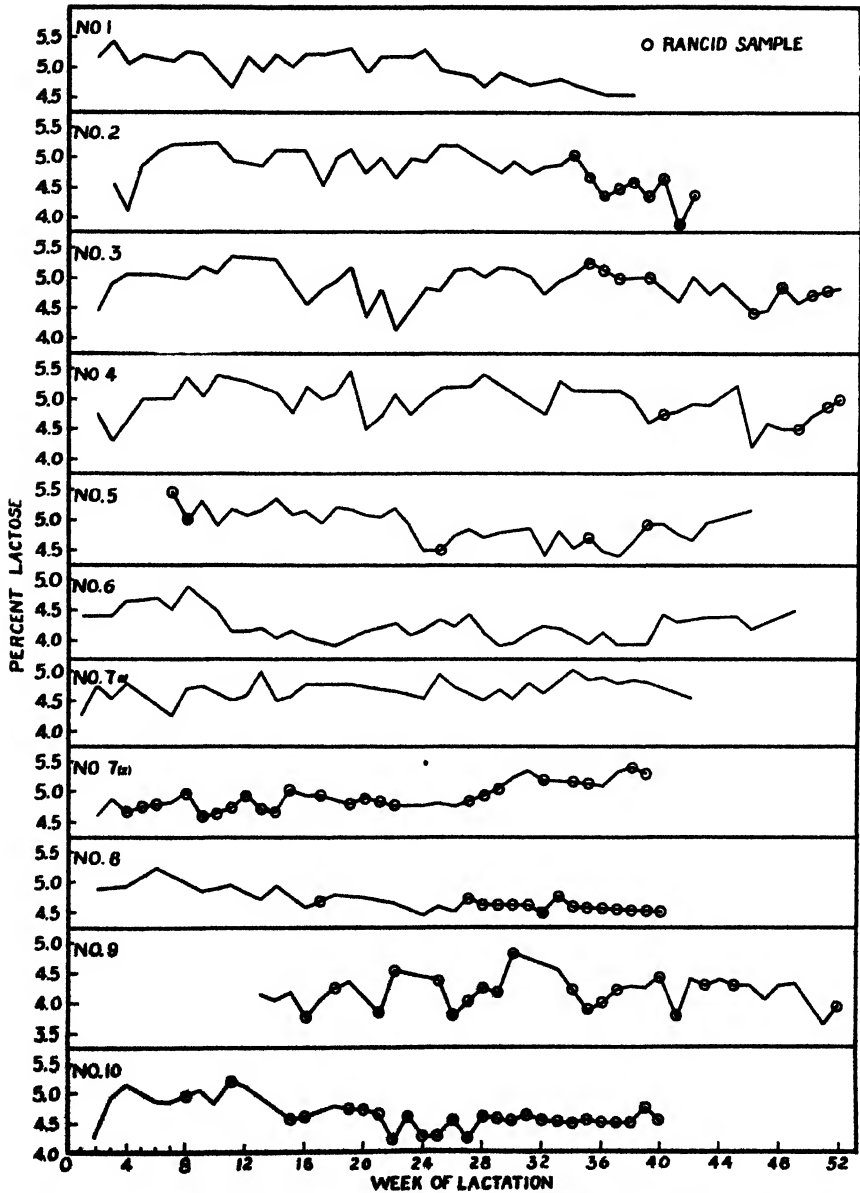


FIG. 8. Lactose content of milk samples taken weekly from each of ten Jersey cows during a complete lactation. Rancid samples are indicated by circles.

TABLE 3  
*Chloride content of rancid, salty and normal Jersey milk*

Description of samples	Milk samples	Chloride content			
		Mean	Standard error	Standard deviations	Coefficients of variation
	<i>number</i>				
"Rancid" .. .	179	0.114	0.001	0.017	14.6
"Salty" .. .	106	0.134	0.002	0.023	16.9
Normal .. .	584	0.098	0.001	0.018	18.5
All samples .. .	869	0.106	0.001	0.022	20.9

<sup>1</sup> The formula used in determining the significance of the difference between the means of the chloride content of normal and "rancid" samples was:

$$\frac{M_R - M_N}{\sigma_d} \text{ in which}$$

$M_R$  = mean chloride of "rancid" samples,

$M_N$  = mean chloride of normal samples,

$\sigma_d$  = standard error of difference =  $\sqrt{(\sigma_R)^2 + (\sigma_N)^2}$ ;

$\sigma_R$  = standard error of mean chloride of "rancid" samples,

$\sigma_N$  = standard error of mean chloride of normal samples.

herd. In Table 1 are found the mean lactose content of the milk of each of the animals represented in Figure 3, together with statistical constants. The coefficients of variation indicate the small degree of variation in the lactose of the milk of an individual animal in the course of a lactation. Individual differences are shown by a range of from 4.11 per cent to 4.97 per cent in the mean lactose content of the milk of the ten cows shown.

The general trend of the lactose content of milk during the lactation period is shown in Figure 2. The graph in this figure represents the average lactose content of all samples of normal milk for each week of the lactation period and was determined by the analysis of 599 samples of milk. As may be seen from the graph, there occurs a slight increase in the lactose content of milk during the first 12 weeks of lactation, followed by a decrease during the succeeding 12-week period, after which the lactose remains constant until a very gradual decrease sets in about the 36th week. The maximum change occurring in the average weekly values was 0.5 per cent.

The above data have been summarized in Table 2 which gives the statistical constants for mean lactose concentrations of all normal samples for each of 12 four-week periods. A comparison of the coefficients of variation for the different periods of lactation with those of individual animals (Table 1) indicates a slightly greater variation in the lactose of the milk of different animals in the same week of lactation than in the lactose of the milk of an individual animal during a complete lactation.

#### *Lactose Content of Rancid Jersey Milk*

The lactose content of naturally rancid samples observed during complete lactations of ten cows is indicated on the individual graphs in Figure 3.

A comparison of the lactose of the normal and rancid milk produced by the same animal shows no difference for samples produced at comparable stages of lactation. The level of the lactose content of the milk of animals frequently producing rancid samples was, however, appreciably lower than that of animals whose milk was very seldom rancid, averaging 4.51 per cent for the frequently rancid milk of cows 8, 9 and 10, and 4.87 per cent for the milk of animals 1 through 5, and 7 (lactation 1), which rarely produced rancid samples.

For the purpose of comparison with the lactose of normal milk in the same period of lactation the lactose content of 154 rancid samples is shown as separate points in Figure 2, in conjunction with the graph showing the average lactose content of the milk of the herd. It is evident from the figure that in most cases the lactose content of the rancid samples was less than the average lactose content of normal milk of the same stage of lactation.

The mean lactose content of 599 normal samples irrespective of the period of lactation was  $4.79 \pm 0.0160$  per cent; the standard deviation of the mean  $\pm 0.3914$ ; the coefficient of variation, 8.17. The mean lactose percentage of 138 rancid samples was  $4.62 \pm 0.0318$  per cent; the standard deviation of the mean  $\pm 0.3733$ ; the percentage coefficient of variation, 8.09.

The mean lactose of rancid samples was significantly lower than the mean lactose of normal samples, the difference between them, 0.17 per cent, being 4.8 times the standard error of the difference,  $\pm 0.0357$ . The lower lactose content of the rancid samples is attributed to the fact that animals whose milk is frequently rancid produce milk having a consistently lower lactose content than do animals whose milk is seldom rancid.

#### *Chloride-Lactose Ratio of Normal and Rancid Jersey Milk*

Roadhouse and Koestler (13) regard the chloride-lactose relation as one of the most important bases of milk taste. They have found that milk samples with a high chloride-lactose ratio are judged less favorably than those of like origin where the ratio is relatively low.

In the present experiment the average chloride-lactose ratio of normal milk was found to remain almost constant throughout the greater part of the lactation period except for an initial drop from 2.6 to 1.9 in the first four weeks of lactation, and a rise from 2.25 to 2.75 after the 42nd week. From the 5th to the 42nd week the ratio showed only a slight increase of from 1.9 to 2.25.

The chloride-lactose ratio of the rancid samples was in most cases higher than the average ratio for normal milk of a comparable period of lactation; fifty per cent of the rancid samples had a chloride-lactose ratio lying between 2.5 and 3.5 when the ratio of the normal samples lay between 1.9 and 2.25. The higher ratio of the rancid samples is the result of the increased chloride and decreased lactose of the rancid samples.

## SUMMARY AND CONCLUSIONS

Data have been presented showing the chloride and lactose content of the milk of animals of a Jersey herd, all of which received the same ration and were exposed to the same environmental conditions. The amounts of these constituents found in milk criticized as rancid have been compared with the amounts present in normal milk produced during the same period of lactation. The data are presented statistically and graphically both for individual animals and for the herd.

Milk from cows frequently producing rancid samples has a higher chloride and a lower lactose content than normal milk produced in the same period of lactation. The chloride-lactose ratio of rancid milk is high. The high chloride and low lactose content appears to be characteristic of all milk produced by those animals whose milk is frequently rancid.

Occasional rancidity occurring in the milk of animals usually producing normal milk, or conversely, the production of normal milk by animals frequently producing rancid samples, cannot be explained on the basis of changes in the chloride and lactose content of the milk.

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# EFFECT OF pH ON THE PRODUCTION OF ACETYLMETHYL-CARBINOL PLUS DIACETYL IN MILK BY THE CITRIC ACID FERMENTING STREPTOCOCCI

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Although the acetylmethylcarbinol plus diacetyl content of milk cultures of the citric acid fermenting streptococci (*Streptococcus citrovorus* and *Streptococcus paracitrovorus*) is normally very low, the adjustment of the pH to the proper point after the bacterial population has increased extensively results in a rapid accumulation of these compounds. Various acids can be used for this purpose (1). Since the organisms reduce acetylmethylcarbinol and diacetyl under favorable growth conditions (2), they may produce the compounds in the absence of added acid and then quickly reduce them while with added acid the reduction is delayed by the unsatisfactory conditions. In a butter culture a pH favorable for the accumulation of acetylmethylcarbinol and diacetyl is established through the production of lactic acid by *Streptococcus lactis* which is present along with the citric acid fermenting organisms.

The work herein reported shows the relationship of different pH values, as produced by various acids, to the production of acetylmethylcarbinol plus diacetyl in milk cultures of the citric acid fermenting streptococci.

## PROCEDURE AND METHODS

For each trial, 300 ml. portions of skimmilk were added to pint milk bottles and sterilized in an autoclave. After the milk had cooled to 21° C., the bottles were inoculated with equal amounts of a milk culture of the organism to be studied and incubated about 24 to 40 hours at 21° C. to permit the development of a large number of bacterial cells. The various cultures were then adjusted to the desired pH values with the different acids, and again incubated for about 48 hours at 21° C. to permit the production of acetylmethylcarbinol plus diacetyl. The acids used were citric, lactic, sulfuric, and 0.15 per cent lactic acid together with the amount of sulphuric necessary to yield the desired pH; the acids were made up as aqueous solutions and sterilized. The acetylmethylcarbinol plus diacetyl in the cultures was determined as nickel dimethylglyoximate per 200 gm. of culture (3). Only one incubation period was used and it is probable that the maximum yield of the carbinol plus diacetyl was not obtained since the content of these materials is not constant in a culture and is either increasing or decreasing, but the incubation conditions were favorable for a high yield and accordingly the results are satisfactory for comparative purposes.

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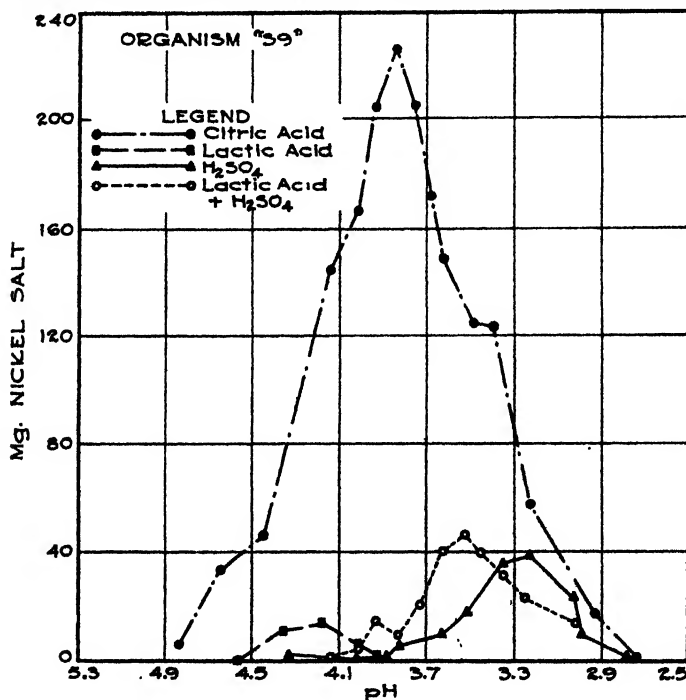
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Project No. 127.

The pH determinations were made electrometrically with a quinhydrone electrode as soon as the acid was added. During the production of carbinol plus diacetyl in a culture the pH commonly increases (1).

#### RESULTS OBTAINED

The data are presented in the form of graphs, each graph giving the results of one trial.

Graph 1 shows data on organism S9. The importance of citric acid as the

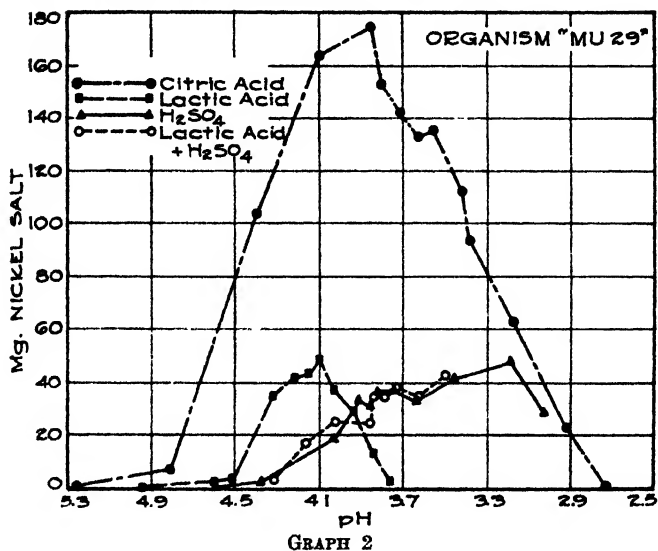


GRAPH 1

source of acetylmethylcarbinol plus diacetyl is evident from the relatively high yields resulting when it was added to the cultures. The maximum yield was obtained at a pH of 3.8.<sup>1</sup> This acid gave a comparatively high yield of the carbinol plus diacetyl over a wide pH range. Lactic acid gave the lowest yields, with the maximum at pH 4.2, and a significant production occurred over a rather narrow pH range. Sulphuric acid gave higher yields than lactic acid and the maximum yield was at pH 3.2; with it the pH range yielding a significant production was wider than with lactic acid. The mixture of lactic and sulphuric acids resembled sulphuric acid alone in the maximum yield of acetylmethylcarbinol plus diacetyl and in the width of the pH range giving a significant production, but the pH at which the maximum yield occurred was 3.5.

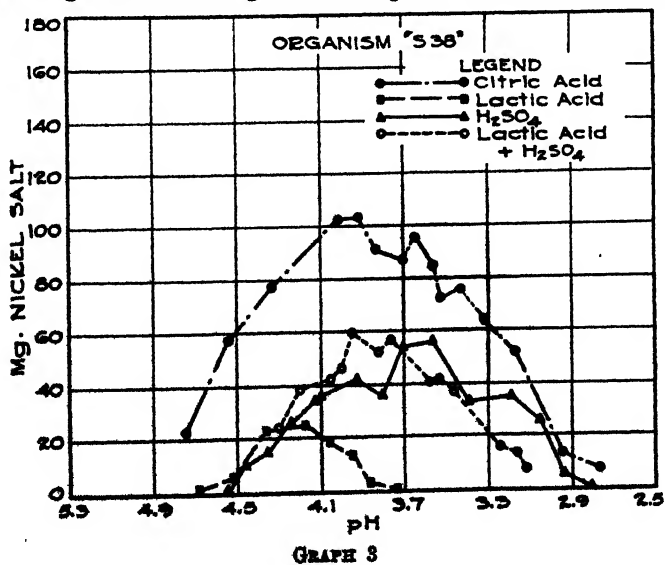
<sup>1</sup> In discussing pH the values given are to the nearest 0.1.

Results on organism MU29 are presented in Graph 2. Citric acid gave a



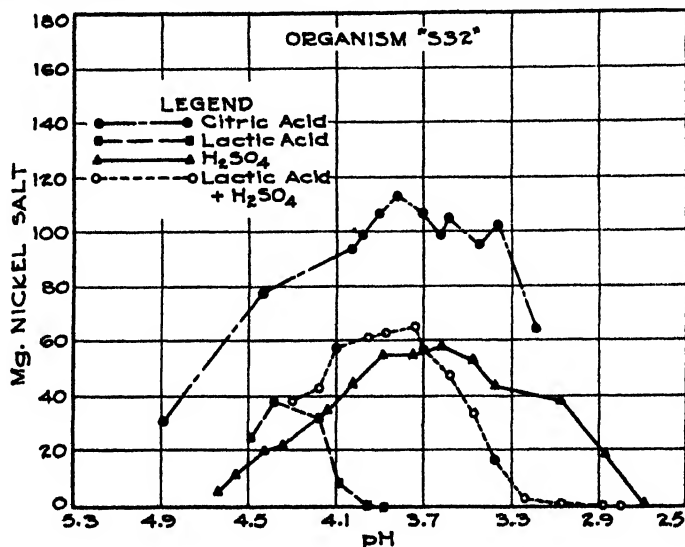
high yield of acetylmethylcarbinol plus diacetyl over a wide pH range with a maximum at pH 3.8. Lactic acid gave a higher maximum yield than with organism S9, the maximum being essentially the same as with sulphuric acid. However, with lactic acid the maximum yield was at a higher pH (4.1) than with sulfuric acid (3.2) and a significant production occurred over a narrower pH range.

Data on organism S38 are given in Graph 3. With citric acid the maxi-

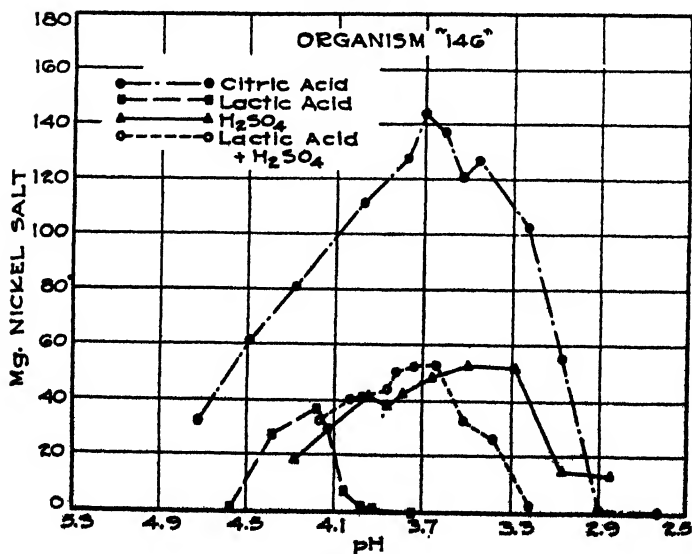


imum yield of acetylmethylcarbinol plus diacetyl, which occurred at a pH of 3.9, was not as high as with the organisms already considered but a significant production was obtained over a wide pH range. Lactic acid gave a relatively low production over a narrow pH range, with the maximum yield at pH 4.2, while sulphuric acid gave a somewhat higher production over a wider range, the maximum being at pH 3.6. The mixture of lactic and sulphuric acids gave a maximum yield at a pH of 3.9.

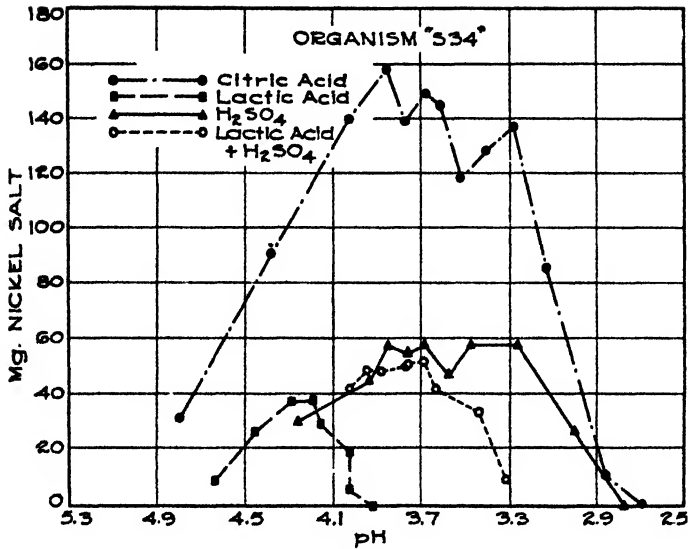
Graphs 4, 5, 6, 7 present data on organisms S32, 146, S34, and DAN2,



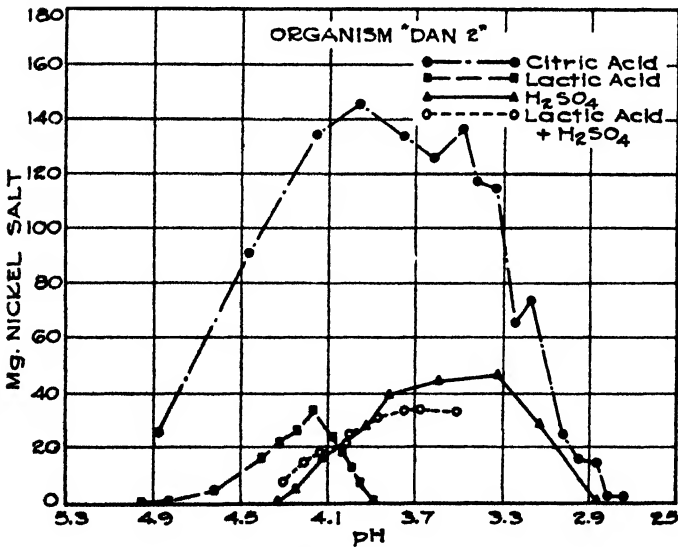
GRAPH 4



GRAPH 5



GRAPH 6



GRAPH 7

respectively. Each of these organisms gave essentially the same results as organism S38. Organism S32 gave the maximum yields of acetylmethylcarbinol plus diacetyl with citric, lactic, sulphuric, and the mixed acids at pH values of 3.8, 4.4, 3.6, and 3.7, respectively; organism 146 gave them at pH values of 3.7, 4.2, 3.5, and 3.6, respectively; organism S34 gave them at pH values of 3.8, 4.2, 3.2, and 3.7, respectively; and organism DAN2 gave them at pH values of 3.9, 4.2, 3.3, and 3.7, respectively.

## SUMMARY AND DISCUSSION

The graphs presented, which represent data obtained on seven cultures of the citric acid fermenting streptococci, emphasize the production of acetylmethylcarbinol plus diacetyl from citric acid. When this acid was used to acidify milk cultures of the organisms, the yield of the carbinol plus diacetyl was always much higher than when other acids were used and a significant production occurred over a wide pH range; the maximum yields were obtained at pH values from 3.9 to 3.7.

With lactic acid as the acidulant, comparatively low yields of acetylmethylcarbinol plus diacetyl were obtained and a significant production occurred over a rather narrow pH range. The maximum yields were obtained at pH values from 4.4 to 4.1 which approximate the pH values commonly found in satisfactory butter cultures. Since lactic acid is the principal acid involved in establishing the pH in butter cultures, the frequent failure to obtain a high yield of acetylmethylcarbinol plus diacetyl in such cultures may be due to the relatively unfavorable character of lactic acid. Sulphuric acid gave higher yields of acetylmethylcarbinol plus diacetyl than lactic acid and also gave a significant production over a wider pH range. The maximum yields occurred at comparatively low pH values which ranged from 3.6 to 3.2. The favorable character of sulphuric acid compared to lactic acid for the production of acetylmethylcarbinol plus diacetyl may explain why the special butter culture made by growing one of the citric acid fermenting streptococci in milk and then acidifying with citric and sulphuric acids (1) are so generally satisfactory. A mixture of 0.15 per cent lactic and variable amounts of sulphuric acid, depending on the pH desired, gave yields of acetylmethylcarbinol plus diacetyl comparable to those obtained with sulphuric acid alone. The maximum yields occurred at pH values from 3.9 to 3.5. In general, these values were between the corresponding values for lactic and sulphuric acids.

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# FLAVOR AND BACTERIAL CHANGES OCCURRING DURING STORAGE OF SWEET CREAM WHICH HAS BEEN FLASH PASTEURIZED AT VARIOUS TEMPERATURES

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The problem of maintaining a desirable flavor in high quality sweet cream involves the control of the bacterial flora in the cream. Since the temperature at which the cream is heated would influence the flora it seemed desirable to study the bacteria which survived the flash pasteurization process. It was hoped that a relationship might be found between the surviving organisms and the final flavor of the cream. The work was undertaken initially as a plant control measure and is offered to others who are interested in similar problems.

Numerous investigators have studied the bacteria which survive in milk pasteurized by the holding method, but very few studies have come to the attention of the author on the high-temperature, short-time or flash process of heating. The work published on the latter method has dealt mainly with thermophilic bacteria (1) in milk or has been limited to other products such as butter. Many of the previous investigations have included studies of the physiologic types of organisms surviving in milk heated for 30 minutes at various temperatures. The method used by Ayers and Johnson (2) to differentiate the various types was to pick individual colonies of bacteria from agar plates and inoculate separate tubes of sterile litmus milk with one colony each; the tubes were incubated for a period of 14 days at 30° C. and the reactions then observed. These investigators divided the organisms into the following groups: acid forming and coagulating, acid forming, inert, alkali forming and peptonizing. Two additional groups, namely, acid coagulating-peptonizing and sweet curdling were added to the above list by Thurston and Olson (3) who employed the "milk-tube" method of Ayers and Johnson in an investigation of high grade milk before and after pasteurization.

In an effort to secure maximum growth of colonies on standard agar, Thurston and Olson incubated their plates 3 days at 19°–21° C., then 2 days at 37° C., before picking the colonies into sterile litmus milk. Several proposals have been advanced to alter the media used in the bacteriological analysis of milk in order to secure more suitable conditions for growth of milk bacteria. The tryptone-glucose-skimmilk agar suggested by Bowers and Hucker (4) was used throughout this study.

Different temperatures of incubation for the agar plates likewise have

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been proposed by others but in this study limited incubator capacity permitted only the use of 37° C. for 48 hours.

#### PROCEDURE

The raw milks which were separated to supply the creams used throughout this investigation were obtained from the daily plant receipts and were representative of approximately twenty thousand pound lots. Samples of the milk were collected in sterile vials at intervals during the separation of the experimental cream and were mixed just previous to plating. About 25 pounds of raw cream testing 44 to 46 per cent fat were obtained directly from the separators and were taken to the laboratory for immediate processing. However, in two experiments the cream was processed in the large barrel-type commercial flash pasteurizer used in the daily plant operation.

Six 100 ml. samples of the raw cream were taken in sterile, cotton-stoppered flasks and placed immediately in ice water. The remainder of the cream was processed by flash pasteurization to 185°, 175°, 165° or 155° F. in the laboratory apparatus previously described by the author (5). The pasteurizer consists of 1-, 1.5- and 2-inch diameter tinned-copper sanitary piping which is telescoped and is fitted with appropriate inlets and outlets for the heating water and the cream. The cream flows through the intermediate section and is heated with hot water which is pumped through the center and outside sections. The temperature of the heating medium was 10° to 15° F. higher than the final temperature of the cream, the latter requiring from 30 to 45 seconds to pass through the pasteurizer. The hot cream was immediately directed over a small tinned-copper surface cooler and was cooled at once to 50° F. Approximately five seconds were required for the cream to flow from the pasteurizer to the surface cooler. The cream heated to 185° F. was pasteurized first and the temperature progressively lowered with adequate time elapsing between collection of the samples so that thorough flushing of the equipment with the cooler cream was assured. Six sterile, cotton-stoppered flasks were filled with the cooled cream from each lot and immersed at once in ice water. All pieces of equipment used in handling the cream were previously scalded for five minutes with boiling water.

The sample flasks of cream were held in the ice-water bath for three hours and were then transferred to a refrigerator maintained at 35° F. One flask of cream from each lot was retained for bacterial and flavor analyses and the procedure repeated every two days thereafter, the final test usually being made when the cream was ten days old. The bacteriological methods followed were the standard procedure (6) with the substitution of tryptone-glucose-skimmilk agar for the standard nutrient media. Duplicate plates were always made for each lot of cream and the dilutions that were necessary to secure plates containing from 30 to 60 colonies were 1:10 and 1:100 for the heated creams while the raw creams occasionally required 1:1000. Fre-

quently the number of colonies found on the plates were considerably above or below the desired limits. All of the plates were incubated at 37° C. for 48 hours before they were counted.

After the samples had been plated the cream remaining in the flasks was warmed to 90° F. and used for flavor analysis; the criticisms were made by two judges.

Immediately after the plates were counted the individual colonies were picked off with a sterile needle and each colony was placed in a separate tube of sterile litmus milk. An effort was made to get from 30 to 60 colonies from each sample but the plates made with the cream which had been pasteurized at 175° and 185° F. usually did not contain that many colonies; in this case all colonies were taken from the two plates. When more than 60 colonies grew on a plate an area containing about that number was chosen and all colonies in that area were picked; selective picking was thus avoided. The inoculated tubes were incubated at 30° C. for 10 days before the final observations were made. The bacteria were divided according to the reactions produced in the litmus milk into the following groups: inert, alkali forming, acid forming, acid coagulating, acid coagulating-peptonizing and sweet curdling.

Incubated tubes of litmus milk frequently were selected from each grouping of bacteria and smears prepared and examined microscopically; the morphologic class of the various organisms was thus determined.

## RESULTS

### *Bacterial Changes*

Twelve experiments were completed during the months from November until the following July, so that milks produced during the winter, spring and summer months were included in the study. In Table 1 are presented

TABLE 1

*Number of bacteria in sweet cream flash pasteurized at various temperatures and stored at 35° F. for several days*

Temperature of flash pasteurization  °F.	Number of bacteria per ml. after storage—days					
	0	2	4	6	8	10
Orig. Milk .....	17,950					
Raw Cream .....	9,296	10,740	15,560	20,140	31,850	97,950
155 .....	1,146	1,217	1,453	1,135	1,242	1,775
165 .....	429	374	461	407	392	495
175 .....	171	188	233	137	185	167
185 .....	115	139	104	98	123	104

the bacterial counts of the flash pasteurized creams during the ten day storage period at 35° F. These counts are averages of the twelve experiments and

were computed logarithmically. The average count of the original raw milks is also included and all of these counts were below 20,000 except one sample each obtained in February, May and July where the counts were 35,000, 40,000 and 73,000. The counts of the raw creams produced from these milks were likewise higher than the others. In the group of pasteurized creams the counts for each range of temperature were quite uniform in all of the experiments although the creams heated at 155° and 165° F. varied more than those heated at 175° and 185° F.

It is evident from the results that the organisms which survive decrease as the heating temperature of the cream is increased. Since more organisms are present in the raw creams than survive in the heated portions the increases in the former, during the storage period, are proportionately greater. The growth curve of the organisms in the raw creams during storage is decidedly upward whereas those of the heated creams are comparatively flat in shape.

The changing picture of bacterial life in the creams is more vividly portrayed in Fig. 1. This chart shows the average percentages of the various

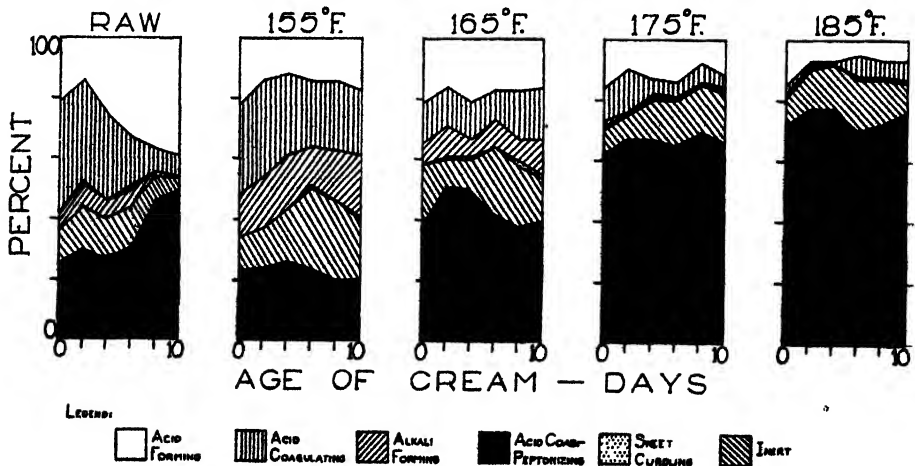


FIG. 1. Changes in the percentage of the various physiologic groups of bacteria which occur in raw and pasteurized sweet cream during storage at 35° F.

physiologic types of organisms surviving in the creams throughout the ten-day storage period and was constructed with the data resulting from the study of the inoculated tubes of litmus milk. In the fresh raw creams the acid coagulating organisms are the largest group, but as these creams are held in storage for ten days at 35° F. the number decreases, particularly after the second day. The acid coagulating-peptonizing and the acid forming groups increase during storage so that after eight days the peptonizers predominate all other groups, although the acid formers are nearly as prevalent.

In the pasteurized creams the acid coagulating group of bacteria are still the largest in those samples heated at 155° F. but do not tend to decrease as

rapidly during storage as in the raw creams. The peptonizing bacteria are reduced as the result of the heat-treatment at 155° F. and do not increase during the storage period. Also a rather large number of alkali forming organisms exists throughout the storage period in the creams heated at the lower temperature. As the heating temperature is raised the percentage of peptonizing bacteria likewise increased; at 155° F. about 25 per cent of the organisms were peptonizers whereas they increased to approximately 40 per cent at 165°, 60 per cent at 175° and 75 per cent at 185° F. This increase of peptonizers was accompanied by decreases in the other groups of organisms which were destroyed by the heat-treatment; the alkali forming group nearly disappeared at 175° F. and above while the acid forming and acid coagulating groups progressively decreased as the heating temperature rose.

The group of inert organisms produce no change in litmus milk; they are present in all samples of cream but represent, mainly, tubes of litmus milk in which the inoculated bacteria failed to grow. It is felt that this group is not an accurate picture of the truly inert organisms, for they were very few in number. If the tubes in which no growth of organisms occurred had produced a growth the final percentages of the other groups would have been altered considerably. Although a few of the acid coagulating group exist at 175° and 185° F. it appeared that a considerable portion of these would peptonize if the tubes were incubated several days more.

The morphologic groups of bacteria which produced the various reactions in litmus milk occasionally were determined microscopically and the observations are presented in Table 2. The table indicates that the acid coagulating

TABLE 2

*Morphologic classes of bacteria from raw and flash pasteurized creams which produced various reactions in litmus milk*

Type of reaction	Classification of bacteria observed
Acid Coagulating	Streptococci—paired and short-chained
Acid Forming	Cocci and bacilli
Acid Coagulating—Peptonizing	Bacilli and cocci
Alkali Forming	Bacilli
Sweet Curdling	Bacilli

organisms were paired or short chained streptococci whereas those which only formed acid were cocci and bacilli. The groups responsible for the acid coagulating-peptonizing type of reactions were likewise bacilli and cocci organisms; the former group also was responsible for the alkali forming and sweet curdling reactions. The temperature at which the cream was pasteurized did not seem to influence the morphologic class of the bacteria which produced a certain type of reaction. An example of this is shown by the acid coagulating-peptonizing bacilli and cocci which were observed in the litmus milk tubes inoculated with colonies from raw cream and from creams flash pasteurized at each temperature studied.

*Flavor Changes*

Associated with the study of the bacterial changes in the cream during storage a parallel study was made of the changes in the predominating flavors of the cream and the results are summarized in Table 3. This table reveals

TABLE 3  
*Changes in the flavor of flash pasteurized sweet cream during storage  
for several days at 35° F.*

Tempera- ture of flash pasteur- ization  °F.	Flavor of cream after storage—days					
	0	2	4	6	8	10
Raw Cream	Good	Slightly Bitter	Bitter	Bitter	Very Bitter	Very Bitter
155	Fine	Very Good	Good	Good	Slightly Old	Old, Stale
165	Slightly Heated	Fine	Fine	Very Good	Good	Good
175	Heated	Heated	Slightly Heated	Slightly Heated	Slightly Heated	Slightly Heated
185	Pronounced Heated	Pronounced Heated	Pronounced Heated	Heated	Heated	Heated

that very undesirable changes in flavor occur in the raw creams soon after separation from the raw milk; after two days storage all of the raw samples, except the two obtained in the summer months, were bitter. The raw cream produced the last week in May retained a fine flavor for six days while that produced the last week in July was slightly stale on the fourth day. The changing seasons of the year accompanied with the changes in the feeds which the cows consume is reflected in the flavors and the ability of the creams to maintain the desirable ones.

The effect of flash pasteurization is to retard these undesirable changes and to prolong the keeping quality of the stored product. A temperature is reached, however, where a heated taste is imparted to the cream, for flash pasteurization at temperatures above 165° F. produced objectionable heated flavors. During the winter and early spring months a pasteurizing temperature of 165° F. generally resulted in producing a cream of the most desirable flavor, whereas the cream produced in the late spring and summer months retained its fine flavors when heated to 155° F. When the cows were on fresh grass pasture in the spring a grassy flavor was imparted to the cream, pasteurization tending to amplify it so that at 165° the flavor was more pronounced than when the heating was limited to 155° F. Creams heated at 175° F. and above usually possessed such a heated flavor that other distinctions were impossible.

Flavor changes occurring between 155° F. and 165° F. in fresh cream are difficult to describe since they are delicate ones to detect. A rich, full, fresh cream flavor is evident when the cream is flash pasteurized at 155° F. whereas at 165° F. the delicate blending of flavors disappears and a harshness or a sharp flavor arises. The higher pasteurization also causes the creams to taste sweeter as though a small amount of sugar, such as lactose, has been added. This apparent increased sweetness is replaced by a sharper heated flavor when the creams are pasteurized at 175° F. and above. Some of the heated flavor detectable in the fresh pasteurized creams disappears upon storage so that many of the samples which had objectionable heated flavors when fresh were classed as very good flavored creams when two to four days old. Creams produced in the late spring and early summer have a more desirable flavor than those produced during the winter months.

Since the study involved numerous separate experiments in which the creams were produced during different seasons of the year it appears that other biological factors in addition to the bacteria no doubt have an important bearing on the flavors of the cream. It is possible that these factors may have been elaborated by the bacteria, but the development of that phase of the problem was beyond the scope of this immediate study.

#### CONCLUSIONS

1. The number of bacteria surviving in sweet cream decreases after flash pasteurization; the reduction occurs inversely to the temperature of heating.
2. The growth of organisms during storage at 35° F. is more rapid in raw than in pasteurized creams. The growth curves in the latter are nearly flat during the ten day storage.
3. In the fresh raw cream the acid coagulating group of bacteria predominates but is gradually replaced by the slower growing peptonizing and the acid forming types as the cream ages.
4. In the flash pasteurized creams the percentages of peptonizing bacteria which survive increases as the heating temperature is raised; this type predominates in the creams heated at 165° F. and above.
5. It is difficult to associate the percentages of the physiologic groups of bacteria present in the various samples with the flavors which occur in the creams; other biological factors evidently play an important part.
6. Very objectionable bitter flavors arise in raw creams shortly after storage.
7. Flash pasteurization at 165° F. prevented the formation of bitter flavors during the 10 day storage period.
8. During the late spring and summer months flash pasteurization at 155° F. produced very fine flavored creams which could be stored for 10 days at 35° F. without objectionable changes in flavor.

9. Flash pasteurization at temperatures above 165° F. imparts objectionable heated flavors to the cream.

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# CONCERNING THE ACCURACY OF THE METHYLENE BLUE REDUCTION TEST<sup>1</sup>

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From time to time discussion has centered about the accuracy of the methylene blue reduction test. Various opinions have been expressed, due no doubt to the fact that different workers have held divergent views as to what the test is supposed to measure.

It is generally accepted nowadays that the decolorization of methylene blue follows the removal of dissolved oxygen from the milk (3, 24, 29, 32). With the ordinary grades of raw milk, this oxygen consumption is believed to be due almost entirely to the respiratory activities of bacteria growing in the milk.<sup>2</sup> Since there is, on the average, a fairly satisfactory correlation between bacterial numbers and reduction time (9, 16, 20, 24, 32), the test has been regarded by some as an index of the number of organisms initially present, from which certain deductions may be made regarding the care exercised in production and handling. On the other hand, those interested in the distribution and processing of milk have been further interested in the test as an index of probable keeping quality, a function which it has been found to perform with reasonable satisfaction (5, 16, 24, 32). The improbability that the test will indicate both numbers *and* keeping quality at the same time with perfect accuracy will be evident from the following:

If the test is regarded purely as a measure of bacterial numbers, then complete accuracy would require that all samples of milk containing the same number of organisms should show identical reduction times. That such perfect correlation will rarely be obtained is evident from the following considerations:

1. Since oxygen consumption is proportional to protoplasmic mass (22), larger cells will generally take up oxygen more rapidly than smaller ones.
2. Different species of bacteria show different reducing intensities (6, 15, 32) and different growth rates at 37° C.
3. Bacteria consume oxygen throughout the lag phase of growth (20, 32); thus a given sample will show a shorter reduction time at the end of this phase although the number of bacteria remains the same.

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<sup>2</sup> In the case of milks with few bacteria, it is believed that leucocytes and other body cells, as well as certain milk constituents, may influence reduction time (13, 24). Certain workers (4, 13) have reported that mastitis milk may reduce more rapidly than would be expected on the basis of the bacterial content, and attribute this to leucocytes. Others (18, 19, 25, 32) have failed to confirm this relationship.

4. The bacteriostatic principle (17) is not of equal influence in all milks or on all species of bacteria (15).
5. Cells dormant from prolonged refrigeration may take longer to reach their maximum growth rate than those not so treated (2, 7).
6. Other milk constituents, as well as body cells, may consume oxygen (13, 24) and influence the reduction time where few bacteria are present.
7. The sweeping of varying proportions of bacteria and body cells from the body of the milk by the rising butterfat often results in variable and delayed reduction times (8, 16, 21, 24, 32).

In addition to these factors affecting the reduction time, it must be borne in mind that none of our present methods for counting bacteria shows a high degree of accuracy (14, 23, 31, 32). Most workers, therefore, would agree with the conclusion reached by Hastings (11) in 1919: "It seems evident from the data submitted that the reduction test determines the number of bacteria as accurately as can be done by any method."

On the other hand, if the reduction test is regarded mainly as an indicator of keeping quality, perfect accuracy would demand exact agreement between reduction time and keeping time.<sup>3</sup> That such will rarely be encountered is evident from the following considerations:

1. The rate of formation of by-products which adversely affect the flavor of milk at 15.5° may not be closely related to the rate of oxygen uptake at 37°. Different types of organisms will tend to predominate at the two temperatures (10, 32).
2. Certain important off-flavors which develop in milk on holding (rancid, bitter, oxidized) are not due to bacterial action.
3. Creaming removes bacteria, leucocytes, etc., from the main body of the milk, as previously mentioned.

Satisfactory comparisons between reduction time and keeping time are also hampered by the difficulty of making accurate determinations of the latter, due to the subjective nature of organoleptic tests. Nevertheless, there is fairly general agreement that "the methylene blue reduction test is as accurate a measure of the keeping quality of milk as any method yet available" (24).<sup>4</sup>

From the foregoing, it will be evident that a certain feature of the reduction test may be regarded as advantageous from the one standpoint, but as the reverse from another standpoint. The sensitivity to metabolic activity dur-

<sup>3</sup> The expression "keeping time" refers to the length of time during which a sample held at 15.5° C. will remain sweet and free from off-flavors and odors.

<sup>4</sup> While Robertson and Frayer (20) found the plate count to be slightly more accurate than the reduction test as an indicator of keeping quality in low count milk, Wilson (32), using a modified technic for the reduction test, reports significantly greater accuracy with the latter than with the plate count.

ing the lag phase of growth is an excellent example. From the standpoint of measuring the true bacteriological condition of the milk, this is a decided advantage, particularly with reference to probable keeping quality. On the other hand, from the standpoint of indicating the *number* of bacteria present, this is a disadvantage, and would be considered a source of error. Consequently, it is believed that the reduction test should be regarded, not solely as a measure of bacterial numbers, but rather as an index of the bacteriological *condition* of the milk, since this would include *activity* as well as *numbers*.

Of the various factors listed above which tend to influence the results of the reduction test there is an important one which can readily be eliminated. Various workers have shown that when the fat (and accompanying bacteria and body cells) is prevented from accumulating at the surface by homogenization, addition of agar or rennet (24, 32), or is redistributed at intervals by inversion or shaking (8, 16, 21, 24, 29, 32), decolorization is uniform, reduction time is usually shortened and marked variations between replicate tubes tend to disappear. This is most noticeable with milks containing few bacteria. Wilson (32) has therefore recommended a modified test, the main feature of which is the inversion of the tubes at half-hourly intervals. This has been adopted in England as the official method for the grading of raw milks. Thornton (28, 29), on the other hand, has recently opposed the adoption of this modification on the grounds that (a) the accuracy of the test is not significantly increased thereby, and (b) the technic is complicated unduly. Although the greater variability of standard reduction times of replicate tubes is freely admitted, the question is raised as to whether such variability is an adequate measure of the relative accuracies of the standard and modified reduction tests.<sup>5</sup>

It should be emphasized at this point that Thornton regards the reduction test purely as a measure of initial bacterial content. As such, he considers that the errors inherent in the test are of such magnitude that the significance of variations in standard reduction times of replicates, or differences between standard and modified reduction times, is difficult to evaluate. Even when the test is regarded in the above light it is difficult to accept the view that the errors introduced by creaming in the standard test are not of sufficient importance to warrant their elimination through a slight change in technic. As previously indicated, however, the test is primarily a measure of bacterial activity as indicated by oxygen consumption. The assumption that such a test is inaccurate unless there is perfect agreement between bacterial activity and bacterial *numbers* appears to be unsound and unwarranted. While it is true that certain workers, particularly those engaged

<sup>5</sup> The term "standard reduction test" refers to the test as officially described in the *Standard Methods of Milk Analysis*, 6th Ed. (1). The term "modified reduction test" indicates that the tubes are inverted at regular (half-hourly or hourly) intervals during incubation.

in official milk control, have tended to regard the reduction time as a quantitative measure of the bacterial content of the milk at the start of incubation, it is believed that the majority would agree with the view expressed by Hastings *et al.* (12) more than 15 years ago: "From many points of view the bacteriologist is chiefly interested in those organisms which are actually growing in milk. It seems probable that the methylene blue reduction test, because it is influenced by these relationships, measures more accurately than does any other method the bacterial activity in the milk."

When the test is regarded as indicating the bacteriological *condition* of the milk, rather than the initial bacterial content, then the basis for Thornton's first objection disappears. As an index of bacterial activity, the modified test undoubtedly possesses a significantly higher degree of accuracy than does the standard test. An accurate determination of the true rate of oxygen consumption is obviously impossible if varying proportions of the bacteria are permitted to concentrate at the surface in the cream layer. Such organisms are unable to affect the oxygen tension of the main body of the milk. The remaining bacteria take longer to consume the oxygen, while there is often irregular decolorization of the dye and poor agreement between replicate tubes. These features virtually disappear when steps are taken to maintain the bacteria in a more uniform state of dispersion. That the small amounts of extra oxygen incorporated in the milk by periodical inversion are of no significance is shown by the excellent agreement between reduction times of homogenized milks by the standard and modified tests (24, 32). It is evident, therefore, that the modified test measures the rate of oxygen consumption with significantly greater accuracy than does the standard test.

In a recent publication (28) Thornton states, "A coefficient of correlation between the results of the standard and modified methylene blue reduction tests calculated for 332 market milks of many classes was found to be  $0.94 \pm 0.004$ . Consequently neither test greatly excels the other in accuracy, despite the greater variability displayed in the standard test which may lead to inaccuracy in evaluating an individual milk." The argument advanced here is obviously refuted by the statement in the final clause. If the standard reduction times for individual samples are variable and inaccurate, as Thornton admits, while modified reduction times are not (8, 16, 24, 29, 32) then the two tests cannot be equally accurate. The high coefficient of correlation reported is entirely beside the point, since this is obtained by averaging the results from 332 different milks. It tells absolutely nothing concerning the relative reliability of single tube determinations on *individual* milks, the point in which the analyst is interested. It is therefore difficult to avoid the conclusion that the modified test, with its extremely close agreement between the individual and mean values for a series of replicates, must be regarded as significantly more accurate and reliable than the standard test.

Frayner (8) has recently reported comparative studies with the standard and modified techniques using 50 to 100 replicate tubes with each method. His results emphasize the inherent unreliability of the standard method. He points out that if single or duplicate tests had been made on these milks by the standard test it would often have been impossible to predict in which of two or more classes they might have been placed. This point deserves more consideration than it has received heretofore; since in routine testing only single tubes are set up, it is highly important that the method employed yield dependable results. A further illustration of the effect of variations in the standard test upon the classification of milks is afforded by the present

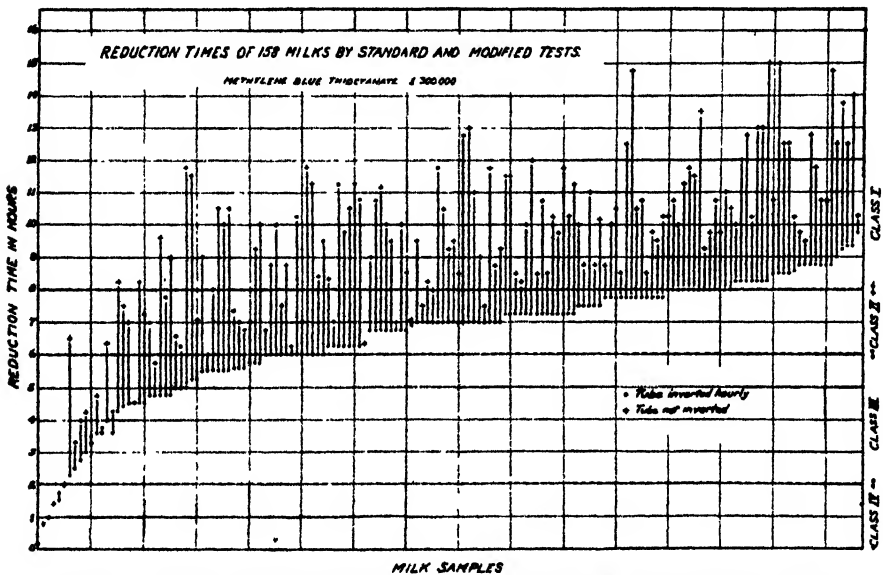


FIG. 1. Illustrating the variability of reduction times by the standard test.

author's data on 158 milks as presented in Fig. 1. Duplicate tubes were incubated together in the absence of light, one of each pair being inverted hourly,<sup>6</sup> the other left undisturbed. Since the modified test furnishes the more accurate and dependable indication of bacterial activity, the modified reduction times have been arranged in ascending order of magnitude and the corresponding standard reduction times plotted against them to show the degree of variability encountered. It will be seen that in the standard test there are wide variations in reduction time for milks showing identical reduction times by the modified test. For example, standard reduction times for the 12 samples reducing in 6 hours by the modified test range all the way from  $6\frac{1}{2}$  to  $11\frac{1}{2}$  hours. Recent findings in this laboratory suggest that such wide

<sup>6</sup> Studies to be reported in a subsequent paper had shown that there was no significant difference in reduction time between half-hourly and hourly inversion. Thornton (29) reports similar findings with 4 samples studied.

differences are not due entirely to the variability shown by replicate tubes in the standard test. As the data in Table 1 indicate, high grade milks from certain herds consistently show greater differences between standard and modified reduction times than do milks of similar quality furnished by other herds. The available evidence indicates that this is associated with the depth of cream layer and the rate of creaming during incubation. It would appear, therefore, that attempts to convert standard reduction times to corresponding modified reduction times according to the equivalent values<sup>7</sup> suggested by Thornton (29) would penalize some shippers while unduly favoring others. It is believed that these inherent differences furnish an added reason for the adoption of the modified test.

In addition to the lengthening of reduction time as milk supplies improve, there are several other considerations which increase the urgency of the need for a more reliable test. The first of these is the proposal to raise the limit for Class I milks from 5½ to 8 hours in the forthcoming 7th edition of Standard Methods of Milk Analysis. Since all investigators agree that the standard test becomes less reliable as the reduction time increases, this extension of the time limits will doubtless result in more serious errors in grading than under the old standards.<sup>8</sup> The reliability of the modified test, on the other hand, is not affected by the lengthening of reduction time. The second is the proposal to increase the dye concentration from 1:700,000 as now employed to 1:300,000 (26). Thornton and Sandin (26) report that with the higher dye concentration, reduction times are lengthened on the average by 30 minutes. Frayer (8) finds an increase in reduction time of the order of 100 per cent with the stronger dye concentration.<sup>9</sup> In the present studies it has been found that the increase is not a fixed increment for all classes of milk as reported by Thornton and Sandin but amounts to about 20 per cent of the reduction time. Data presented in Fig. 2 from comparative tests with single tubes inverted hourly illustrate this. While there are some exceptions, the general trends of the two curves show good agreement, the spread between the two sets of values widening as the reduction time lengthens. Since the bacteria were maintained in fairly uniform dispersion by hourly inversion, the occasional departures from the average are unlikely to be due to creaming.

<sup>7</sup> The equivalent values suggested are:

<i>Standard reduction time</i>	<i>Modified reduction time</i>
8:00	6:00
5:30	4:00
2:00	2:00

<sup>8</sup> In expressing the view in 1930 (16) that the standard reduction test was reasonably accurate up to 10 hours, the author had in mind its accuracy compared with that of the plate count in the examination of better class milks. Thornton (29) apparently holds the same opinion. It cannot, however, be regarded as equivalent in accuracy to the modified test beyond the first few hours.

<sup>9</sup> As subsequently mentioned, the technic adopted by Frayer may be responsible for the unusually great increase reported.

TABLE 1  
Standard and modified reduction times of consecutive samplings from certain herds

Shipper's number	S	M	S	M	S	M	S	M	S	M	S	M	S	M	Ratio S M
1 N	12:15	6:45	15:00	8:30	9:15	6:00	8:45	6:15	15:00	7:30					1.72
1 P	6:30	4:00	11:45	6:00					6:45	5:30					1.61
2 N	10:00	5:30	10:15	5:00	10:00	5:45	8:15	4:00	9:15	5:00	6:45	5:30			1.77
2 P	8:30	4:00	3:15	2:40	6:45	3:30			4:45	2:45	8:00	4:40			1.78
3 N	15:00	8:15	13:00	6:45	12:45	7:00	10:15	6:45	11:30	8:30					1.68
3 P	10:10	6:30	8:15	5:15	12:00	5:15			12:00	7:00					1.57
4 N	13:00	8:15	11:45	5:30	11:45	7:00	10:45	5:45	11:15	6:15	10:00	6:00	14:00	4:40	1.90
4 P	3:15	2:40	1:40	1:30	2:50	2:00	1:20	1:20	8:15	3:45	2:30	2:00	2:00	2:00	1.49
5 N	11:15	6:15	10:15	5:00	10:45	6:00	9:15	5:15	11:00	7:00	11:00	6:45	7:45	4:15	1.76
5 P	3:45	2:00	1:30	1:20	2:50	2:10	1:10	1:10	7:00	4:00	2:00	2:00	2:00	1:30	1.50
6 N	8:00	7:00	5:15	4:15	8:30	7:00	5:45	4:45	10:45	8:45	12:00	10:15	6:30	5:15	1.20
6 P	4:45	3:45	2:40	2:40	7:00	4:45	2:50	2:10	7:15	5:00			5:30	3:00	1.40
7 N	6:15	6:00	7:15	5:30	8:30	8:00	8:00	7:00	9:15	8:45	7:00	6:15	3:15	3:15	1.11
7 P	1:20	1:20	1:00	0:50	5:30	5:00	4:00	4:00	4:45	4:45			1:00	1:00	1.05
8 N	6:45	6:00	7:00	6:45	8:15	7:15	9:30	7:30	9:45	8:00	8:30	7:15	7:15	5:00	1.19
8 P	3:30	3:30			4:10	4:30	2:40	2:00	4:00	3:45	7:30	5:15	4:45	3:00	1.20
9 N	8:15	7:00	7:30	6:15	8:30	7:45	7:15	6:30	13:45	9:15	6:00	5:15	2:30	2:15	1.21
9 P	3:45	3:40	2:00	2:00	6:15	5:15			10:15	7:15	1:00		1:00	0:45	1.23

N.B. S = Standard Reduction time. M = Modified Reduction Time. N = No Preliminary Incubation. P = Preliminary Incubation at 12.8° C. for 18 hours.

It is more probable that differences in dye sensitiveness of the milk flora may be the cause.

At this point, mention should be made of some anomalous results encountered in 1934 when collaborating with Dr. H. J. Conn, Chairman of the Commission on Standardization of Biological Stains, in the testing out of the new methylene blue thiocyanate. With certain samples the modified test led to significant lengthening of the reduction time with the higher dye concentrations. This was not noted where the standard test was employed.<sup>10</sup>

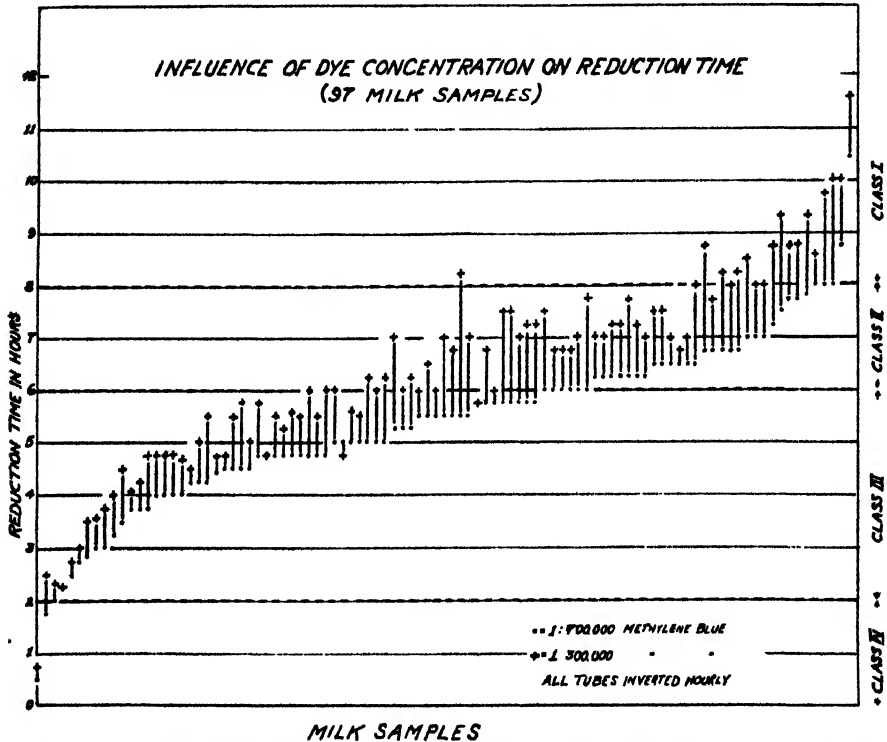


FIG. 2. Corresponding reduction times of 97 milk samples with old and new dye concentrations.

Similar cases have been encountered only twice during the present studies covering several hundred samples. Such cases would appear therefore to be too infrequent to deserve special consideration.

In passing, attention should be called to the fact that there is a much more satisfactory agreement between the modified reduction times with the two different dye concentrations (Fig. 2) than is the case with the standard and modified reduction times at either dye concentration (Fig. 1).<sup>11</sup> It will

<sup>10</sup> Thornton (30) has encountered two samples giving similar results.

<sup>11</sup> Results almost identical with those shown in Fig. 1, were obtained where a dye concentration of 1: 700,000 was employed.

also be noted that with the exception of Class IV milks the increase in reduction time resulting from the stronger dye concentration is considerably less than the average increase where creaming is permitted to take place. The mixing modification therefore more than compensates for the delayed reduction with the stronger dye concentration.

A further reason advanced by Thornton (29) for rejecting the modified test is that "it complicates the operation of the test to the extent that strictly uniform technic would be improbable in the hands of some who are at present satisfactorily performing the test." This was originally interpreted as referring to the *technic* of inverting the tubes but in commenting on the original draft of this paper Thornton (30) indicated that he had in mind (a) the difficulty of getting operators of the test, who often have various other duties to perform, to invert the tubes every hour, and (b) the test will have doubtful application in cheese factories, etc., if it is made the least bit more complex. Concerning the first point, it has been found in the present studies that any program of inversion, no matter how irregular the intervals, will increase the reliability of the test.<sup>12</sup> The slight inconvenience involved in periodical inversion of the tubes appears to be more than compensated for by the shortening of reduction time, simpler end-point determination, and greater reliability with the better grades of milk. As to the second point, the mixing modification is least valuable with the poorest grades of milk. For the ordinary run of cheese milks it will probably make little difference if inversion is omitted. On the other hand, where the test is used as the basis for premium payments for the best milks,<sup>13</sup> the more accurate technic should be employed. In the interests of uniformity and accuracy, therefore, it would seem desirable that the modified test replace the present technic for all classes of milks.

#### TECHNIC OF DETERMINING THE END-POINT IN THE MODIFIED TEST

Most investigators have recognized that reincorporation of oxygen immediately prior to reduction may unduly prolong the reduction time of certain milks and have modified their technic accordingly. Wilson (32, p. 216) specifies that "Any tube at the time of examination showing obvious signs of reduction should not be inverted, but should be left until the end-point as defined is reached." Thornton (30) employs a similar procedure, as does the present author. Frayer (8) does not state his procedure. That some judgment must be exercised by the operator of the test in deciding when to stop inverting the tubes is admitted. It is believed, however, that the difficulties involved are no greater, and probably far less, than those involved

<sup>12</sup> Thornton's own data (29, Table 6) show marked improvement resulting from inversion of tubes at the 4th, 6th and 8th hour of incubation.

<sup>13</sup> A program for improving cheese milk supplies along these lines is being considered at present in Wisconsin.

in attempting to estimate the end-points of the majority of better-class milks under the standard technic, where patchy and uneven decolorization of the dye is often encountered.

The method of determining the exact end-point with the reduction test is deserving of further study. Most investigators have worked out a technic for reading the end-point which satisfies them. That employed by the author is similar to that described by Wilson (32, p. 231), who remarks that "the point of 'complete reduction' of methylene blue is impossible to measure either visually or electrometrically." Frayer (8) on the other hand states that he has taken the point of complete reduction by transmitted light as the end-point but gives no details of his method. It seems not unlikely that this may be responsible for some of the differences between his results and those of other workers in comparing different dye concentrations, particularly if tubes were inverted without regard to incipient reduction.

#### SUMMARY

1. The methylene blue reduction test is based upon the rate of oxygen consumption in the milk by the bacteria present during incubation. This rate cannot be measured with reasonable accuracy where varying proportions of the bacteria are removed from the main body of the milk during the creaming process.

2. The accuracy of the test is greatly increased by periodical inversion of the tubes during incubation. In addition, reduction time is generally shortened and decolorization is more uniform, especially with the better grades of milk.

3. It is believed that these advantages more than compensate for the slight inconvenience entailed by this modification.

4. Milks showing similar reduction times by the modified technic sometimes show wide differences in standard reduction times. These appear to be associated with differences in the degree and rate of creaming.

5. Reduction time is prolonged by approximately 20 per cent with the proposed stronger dye concentration. This is more than compensated for in all but the poorer grades of milk by the shortening of reduction time when inversion is practised.

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# THE INFLUENCE OF ACIDITY VARIATIONS DURING MANUFACTURE ON THE QUALITY AND RATE OF RIPENING OF BLUE OR AMERICAN ROQUEFORT CHEESE

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The limits of desirable acidity development at the various stages in the manufacture of cheddar cheese have been established by the results of numerous investigations. Comparatively little of such information is available to the manufacturer of Blue or American Roquefort cheese. No mention is made of acidity control in the reports of the methods used in France in the manufacture of Roquefort cheese (1, 2, 3). Funder (4), working in Norway on a modification for making Roquefort type cheese from cow's milk, recommended the addition of 2 to 2.5 per cent culture in order to secure mold vegetation. He considered a high acidity necessary for abundant growth of the penicillium.

Thom and Matheson (5) have given the subject considerable attention. According to their experiments cow's milk should be brought to an acidity of about 0.23 per cent by the addition of starter or by the use of a smaller amount of starter with a subsequent ripening period, before the addition of the rennet. The cheese from milk set at a lower acidity did not drain properly, were soft and developed off-flavors. Higher setting acidities were found to be detrimental to the texture of the curd; as tough, waxy or gummy textures frequently developed.

Matheson (6) later again recommended that the milk be set at an acidity of 0.21 to 0.23 per cent. Goss, Neilson and Mortensen (7), however, state that the rennet should be added when the milk reaches an acidity of 0.19 to 0.20 per cent. The actual amount of acidity due to added starter and to ripening, which doubtless is of more importance than the acidity of the milk itself, is not mentioned.

## EXPERIMENTAL

In the manufacture of Blue cheese at the Dairy Division of the University of Minnesota, considerable variation in the rate of ripening of this cheese has been noted. Along with other factors which might influence the rate of ripening and the uniformity of the cheese, acidity variations during manufacture have been investigated. Variables studied included the amount of starter used, the extent to which the milk was ripened and the acidity of

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the whey at dipping. The starter used was a culture of *Streptococcus lactis* with associated types.

In each of 5 replicated series, 4800 pounds of raw milk were used. The milk was divided into six lots. Three lots in each series were set at approximately the acidity to which the addition of 4 per cent starter would bring the milk. The other 3 lots were permitted to ripen, following the addition of starter, to an acidity about 0.03 to 0.04 per cent higher. The initial acidity of the milk in the different series varied from 0.15 to 0.20 per cent, causing an equivalent variation in the acidity at which the milk in the comparable lots in the different series was set. In most cases the milk in the low acidity group was set at 0.19 to 0.20 per cent and the high setting group at 0.23 to 0.24 per cent acidity. In each group of 3 vats, one vat was inoculated with 2 per cent starter, one with 3 per cent and the other with 4 per cent starter. Each vat was divided with respect to dipping acidity, half the vat being dipped soon after cutting, while the remainder was left in the whey until the acidity had increased 0.05 to 0.06 per cent. This required about one hour in most cases.

Every effort was made to standardize the composition of the cheese, particularly the salt content. The cheese were salted by rubbing salt over their surfaces. In three applications a total of 7.5 pounds of salt per 100 pounds of green cheese was used. This procedure has been found at this station to produce uniformly cheese containing about 5.5 per cent of salt at 3 months of age.

In addition to the customary manufacturing data, pH determinations were made at the various stages during manufacture and ripening. To follow the cheese ripening, analyses were made for volatile fatty acids and amino nitrogen on the 2-day-old cheese and on the cheese at 3, 6 and 9 months. The cheese were examined for flavor, body, texture, and character of mold growth at 3 month intervals. The cheese also were analyzed for moisture, salt and fat. The pH determinations were made using a quinhydrone electrode with a Leeds and Northrop type K potentiometer. The method described by Currie (8) for the estimation of volatile acids was followed. Results are expressed as millimeters of N/10 acid per 100 grams of cheese. The amino nitrogen values were determined using the micro-Van Slyke gasometric method. Results are expressed as milligrams of nitrogen per gram of dried fat free cheese. One half cheese was ground and used for analysis at each period. Since the cheese are handled separately, individuality of the cheese assumes a considerable role in the variability of the data.

## RESULTS

Only one important difference was observed in the cheese during the manufacturing period. The customary procedure is to remove the cheese

from the hoop after about 20 hours. In the first series the cheese made from the milk to which 2 per cent starter was added and set at low acidity were so soft that they flattened somewhat on removal from the hoop. This flattening has been observed in other cheese set at low acidity, particularly in instances in which an inactive starter was used. These results indicate that 2 per cent starter is about the minimum which should be used unless the milk is to be ripened considerably.

The amount of starter used does not appear to have significantly influenced any character of the cheese. This is in keeping with the results secured by Thom and Matheson (5) who found that it was immaterial whether the desired acidity was secured by the addition of starter alone or in part by ripening the milk subsequent to the addition of a smaller amount of starter.

TABLE 1

*Influence of variation in setting and dipping acidity on the pH of the cheese at various intervals during ripening*

Acidity during mfg. of cheese	Average pH of cheese at different stages of ripening			
	salting	3 months	6 months	9 months
Low setting	4.76	6.24	5.98	5.82
Low dipping				
Low setting	4.72	6.00	5.89	5.71
High dipping				
High setting	4.79	6.04	5.92	5.63
Low dipping				
High setting	4.74	5.98	5.86	5.60
High dipping				

### *Hydrogen Ion Concentration of Cheese*

The acidity of the cheese as indicated by the hydrogen ion concentration appears to have been influenced at certain stages in the ripening of the cheese both by the acidity of the milk at setting and by the acidity of the whey at dipping. These results are summarized in Table 1. Examination of the data by analysis of variance shows that at salting there were no significant differences in the pH values of the cheese in the different acidity groups. At 3 and 6 months the average pH value for the cheese set and dipped at low acidity is somewhat greater than for the other cheese. Those cheese set and dipped at high acidity have the lowest average pH value. These differences are not statistically significant as the ratio of the greater to the lesser mean square is 3.5 at 3 months and 2.2 at 6 months. According to Snedecor (9) unless the value of  $F$  (ratio of the greater to the lesser mean square) for this number of observations is equal to or greater than 4.20 there are more than five chances in a hundred that there is no real difference in the data.

By the time the cheese were 9 months of age, however, variations in the setting acidity and in the dipping acidity appear to have caused significant

differences in the acidity of the cheese. The cheese set and dipped at low acidity have the highest and those set and dipped at high acidity the lowest average pH values. The ratio of the greater to the lesser mean square is 22.2 which is far outside of the range of Snedecor's highly significant value, which for this number of observations is 7.64. This indicates that there is much less than one chance in a hundred that there is no real difference in the data.

With high setting acidity, variation in the dipping acidity did not significantly influence the pH of the cheese at 9 months. The difference in the average pH values for the cheese dipped at high and low acidities in the low setting acidity group, however, is highly significant. Setting acidity appears to have affected the pH values of the cheese at this period irrespective of whether the dipping acidity was high or low. The difference, however, is slightly greater for the low dipping acidity group.

### *The Rate of Ripening*

A summary of the data on fat hydrolysis and protein degradation is shown in Figure 1. The values shown are the means for the 60 cheese at each period of analysis. The increase in the volatile fatty acids and amino nitrogen with time is apparently essentially a straight-line relationship.

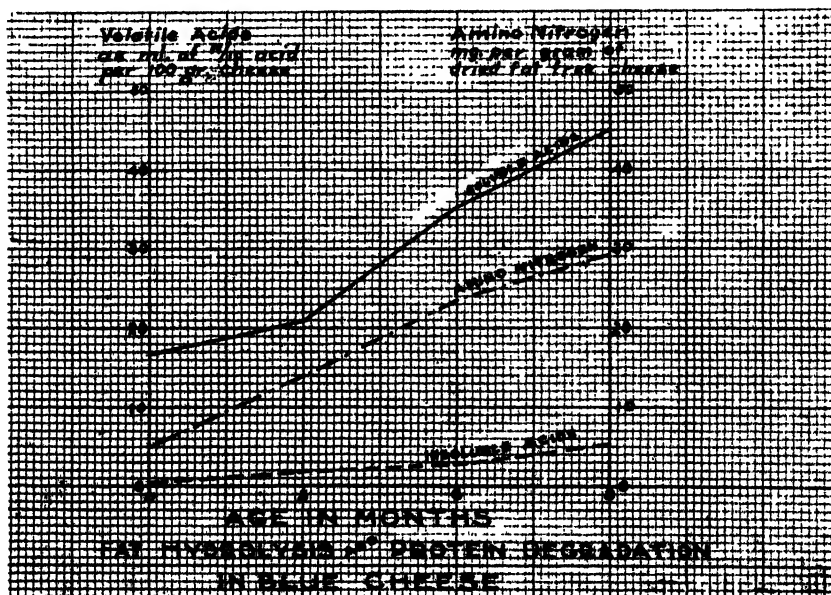


FIG. 1.

The mean values for soluble and insoluble volatile acids and amino nitrogen grouped according to setting and dipping acidity are shown in Table 2.

A low dipping acidity especially when combined with low setting acidity appears to favor fat hydrolysis and protein degradation. The differences between the values for those cheese for which the curd was dipped at low acidity and those dipped at high acidity in the low setting acidity group are significant or highly significant as shown by the values for F, except for the amino nitrogen values at 6 and 9 months and the soluble volatile acids at 3 months. The same trend is apparent at these periods, although the values of F are not sufficiently great to indicate a significant difference.

The mean values for volatile acids and amino nitrogen for the cheese dipped at low acidity are also higher than those for the cheese dipped at high acidity in the high setting acidity group. The F values indicate, however, that there are somewhat more than five chances in a hundred that these differences are due to chance.

For those cheese for which the dipping acidity was high, whether the milk was set at low or at high acidity, there appears to have been no effect on the rate of ripening. The mean values for volatile acids and amino nitrogen are similar at every period. A faster rate of ripening appears to have occurred in those cheese set at low acidity than in those set at high acidity when the dipping acidity was low. These differences are not large, nor very uniform, but do indicate a trend.

#### *Relation of pH to Rate of Ripening*

Considering the data as a whole there does not appear to be any consistent difference in the amount of soluble and insoluble volatile acids and amino nitrogen at the various periods attributable to differences in the hydrogen ion concentration of the cheese. As noted previously only at 9 months was there any significant difference in the pH of the cheese in the various acidity groups. At this period the mean pH of the cheese set and dipped at low acidity was very significantly higher than the mean pH of those set and dipped at high acidity. At this period also, the volatile soluble acids and amino nitrogen values for the group set and dipped at low acidity were very significantly higher. The mean value for volatile insoluble acids was higher but not significantly so. The correlation coefficient between the pH and amino nitrogen values for the cheese in these groups at this period is  $+0.82 \pm .06$ , between pH and volatile soluble acids  $+0.69 \pm .096$ , and between pH and volatile insoluble acids  $+0.29 \pm .17$ . Thus there seems to be a definite association between pH and amino nitrogen and pH and volatile soluble acids in the cheese at 9 months. Whether the volatile acidity and amino nitrogen are low because ripening was retarded by low pH, or whether pH is high because of changes in pH due to decomposition products, it is impossible to say.

TABLE 2

*The influence of high and low dipping acidity and high and low setting acidity on fat hydrolysis and protein degradation of Blue cheese*

Acidity at Dipping	Volatile Acids						Amino Nitrogen		
	Soluble			Insoluble					
	3 mo.	6 mo.	9 mo.	3 mo.	6 mo.	9 mo.	3 mo.	6 mo.	9 mo.
	ml.	ml.	ml.	Low Setting Acidity			ml.	mg.	mg.
Low	21.87	41.43	57.29	2.25	3.39	6.03	15.65	24.60	30.85
High	17.60	32.21	39.31	1.60	2.63	4.61	13.86	23.53	29.24
Value of F.*	3.30	10.51	11.30	4.17	7.24	8.52	6.34	1.68	2.27
High Setting Acidity									
Low	23.37	36.48	44.52	2.13	3.17	5.20	14.05	23.64	28.46
High	21.27	32.33	39.24	2.01	2.68	4.96	12.20	22.50	27.64
Value of F.*	1.20	2.59	1.98	0.22	2.07	0.12	4.09	1.28	0.67

\* For this number of observations (30) a value of F. as great as 4.20 indicates a significant difference, a value as great as 7.64 a highly significant difference [Snedecor (9)].

### *Quality of Cheese*

Variation in acidity during manufacture did not significantly influence the grade of the cheese but did influence to some extent the occurrence of certain flavors in the cheese. In general high acidities during manufacture resulted in more acidy flavored but less musty flavored cheese. Firm bodied and crumbly cheese occurred more frequently where high acidities were used. The cheese set and dipped at low acidities were ready for market somewhat sooner than those set or dipped at high acidity. This would be expected considering the effect of low dipping and low setting acidity on the rate of fat hydrolysis and protein degradation. These cheese, however, deteriorated in flavor sooner. The cheese in the manufacture of which high acidities were used ripened later, but many of these were sold as excellent cheese when over one year old.

### SUMMARY AND CONCLUSIONS

Blue cheese were manufactured using 2, 3 and 4 per cent starter, with the milk set at low acidity (0.19 to 0.20 per cent), and after ripening to 0.23 to 0.24 per cent, with the curd dipped after cutting before additional acidity development, and with dipping delayed until 0.05 to 0.06 per cent acidity developed in the whey.

Variations to this extent in acidity during manufacture do not appear to be highly important in the manufacture of Blue cheese. Excellent cheese were produced with all combinations used. Cheese set and dipped at low acidity as indicated by fat hydrolysis, protein degradation and character of the cheese, ripened somewhat sooner than those set and dipped at high acidity.

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## American Dairy Science Association Announcements

### AMERICAN DAIRY SCIENCE ASSOCIATION ANNUAL MEETING JUNE 14-17, COLUMBUS AND WOOSTER, OHIO

#### HOUSING PLANS

The University girls' dormitories located on the campus will be available beginning June 14 for approximately 350 people at a cost of \$1.00 per person per night. The rate for children will be the same as for adults. Many of the rooms have connecting baths and all facilities are good. The dormitories are within one block of the place of meetings. Rooms will also be available at special rates in Columbus hotels. Members will receive full details regarding housing in a letter to be sent out in early May. All reservations and further information on housing can be obtained from the chairman of the Housing and Registration Committee, J. H. Erb, Dept. of Dairy Technology, Ohio State University, Columbus, Ohio.

#### GOLF AT THE CONVENTION

It is hoped that those in attendance at the Annual Meeting will take advantage of the excellent golf facilities at the University. The University is opening a new course this spring which will be available at a nominal cost.

Make your plans now to bring your family and attend the annual meeting.

#### JOURNAL CIRCULATION

The officers of your Association are attempting to increase the circulation of the JOURNAL OF DAIRY SCIENCE to 2000.

In 1936 the officers made an agreement with the International Association of Milk Dealers and the International Association of Ice Cream Manufacturers to publish abstracts of literature on milk and milk products. The two Associations agreed to promote the circulation of the JOURNAL. The circulation was increased a little more than 400. It reached about 1785 in 1936.

In 1937 there were 958 members, 745 subscribers and 111 associate subscribers making a total circulation of 1813.

Each year we have about 300 who drop their membership or subscriptions. In order to get our circulation to 2000, it will be necessary to increase the membership and subscribers by about 500.

By the fifteenth of March, 790 of the 1937 members had paid their dues, 539 of the subscribers had renewed, and 70 of the associate subscribers had renewed for 1938. Many of the delinquent members and subscribers will, we trust, pay up in 1938. We now have 107 new members, 82 new subscribers, and 49 new associate subscribers, making a total circulation of 1637.

We will appreciate your requesting subscription order blanks or application blanks to send to prospective members and subscribers.

We should have more commercial breeders and those interested in dairy production as our readers. It will be necessary to increase our circulation to carry the additional cost of production abstracts.

We are sorry to announce the passing of Prof. Rush B. Locke on January 27, 1938. Professor Locke had charge of the work in dairy manufacturing at the Colorado State College, Fort Collins, Colo. He had been ill for the past six months.

Prof. Rush B. Locke was born April 22, 1898. He attended South Dakota State College from which he received the bachelor of science degree in June, 1924. In 1925 and 1926 he occupied a teaching fellowship at Iowa State College from which institution he received the degree of master of science in 1926. From 1926 until 1928 he was an instructor in the Department of Dairy Manufactures at Iowa State College. In 1928 he came to the Colorado State College as associate professor of dairy manufactures.

In August, 1937, he went to Fitzsimmons General Hospital (U. S. Army), Denver, Colorado, for observation. He remained there almost continuously, with the exception of a few happy week-ends at home, until his death January 26, 1938, when he died of nephritis and heart disease. Surviving him are Mrs. Locke and their three sons, Rush, Jr., 11 years of age, Richard, 9, and David, 7. Locke was much admired and respected by his friends on our campus and had played an especially important role by establishing and managing a dairy short course of several days duration each year.

# JOURNAL OF DAIRY SCIENCE

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## THE CHEMICAL COMPOSITION AND PROPERTIES OF NORMAL AND RANCID JERSEY MILK

### II. FAT, TOTAL SOLIDS AND PROTEIN CONTENT

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In a previous paper (6) dealing with milk flavor as related to composition, the chloride and lactose content of rancid milk was compared with that of normal milk from a selected group of Jersey cows. The present paper presents the fat, total solids and protein content of the normal and rancid milk produced by these cows.

Because of the recognized variation in the composition of milk due to such factors as individuality, environment and management, available data could not be used as normal values for the herd under observation. In order to make a direct comparison of the composition of normal and rancid milk at all stages of lactation it was necessary to establish normal values for individual cows and for the whole herd for the entire lactation period. This has been done and the results are reported below.

#### EXPERIMENTAL

A representative sample of the evening milk from each cow in the herd was taken at weekly intervals. Milk fat and total solids were determined on the Mojonnier. Total protein, casein and albumin were determined by the official methods outlined in the Methods of Analysis of the A. O. A. C. Management of the animals and the method of sampling have been described in a previous paper.

#### PRESENTATION OF DATA

##### *Fat Content of Normal Jersey Milk*

Weekly changes in the fat content of milk from ten cows during a complete lactation are shown by graphs in Figure 1. It is evident from the graphs that the fat of the milk of the individual animal fluctuated appreciably from week to week. The degree of this variation is shown in Table 1

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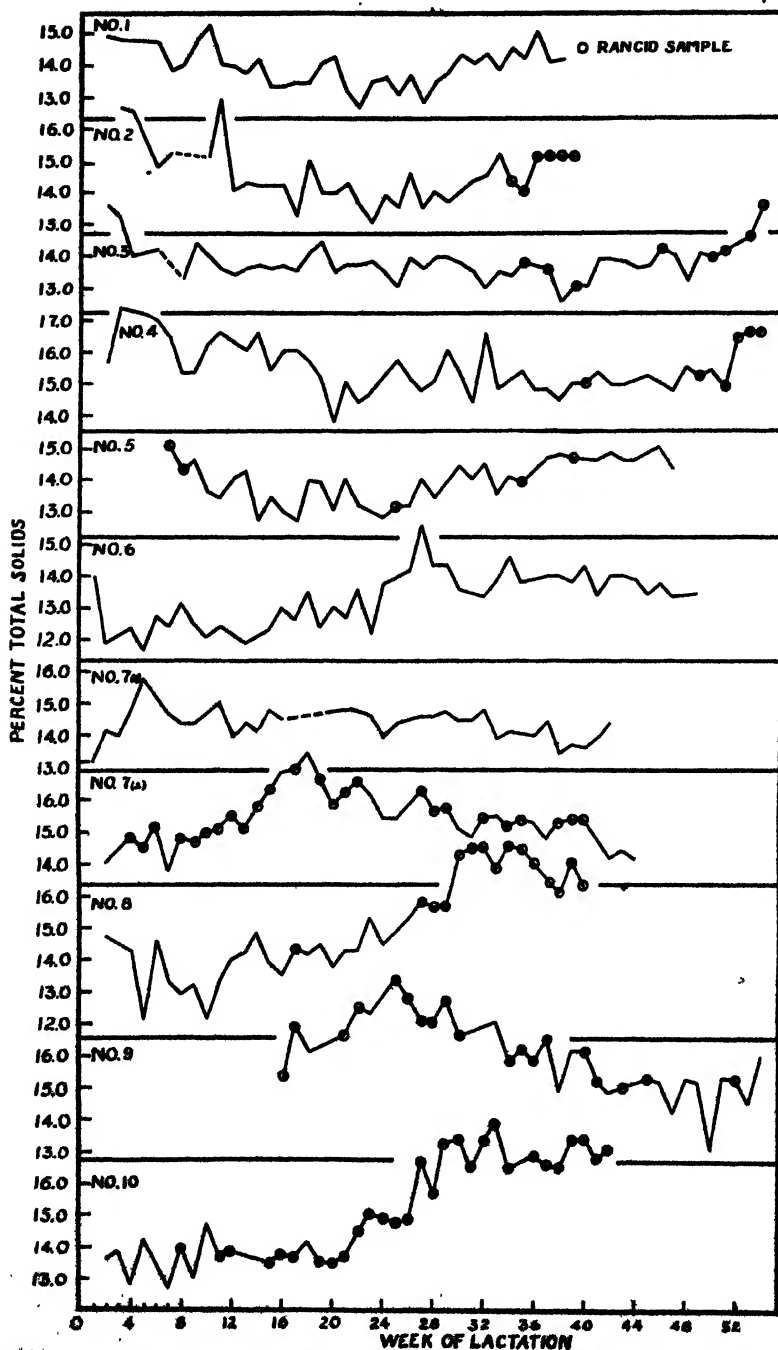


FIG. 1. Fat content of milk samples taken weekly from each of ten Jersey cows during a complete lactation. Rancid samples are indicated by circles.

TABLE 1  
Fat and total solids content of normal and rancid Jersey milk produced by ten<sup>1</sup> Jersey cows during a complete lactation

Cow	Milk samples		Fat content				Total solids content					
	Total	Rancid	Mean of normal samples	Mean of rancid samples	Mean of all samples	S. D. <sup>2</sup>	C. V. <sup>3</sup>	Mean of normal samples	Mean of rancid samples	Mean of all samples	S. D. <sup>2</sup>	C. V. <sup>3</sup>
number	number	number	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
1	36	0	4.81		4.81 ± 0.08 <sup>4</sup>	0.48	10.0	13.96		13.96 ± 0.11 <sup>4</sup>	0.67	4.8
2	34	6	5.16	5.12	5.16 ± 0.12	0.67	13.1	14.45	14.77	14.50 ± 0.16	0.91	6.3
3	49	9	4.56	4.95	4.61 ± 0.08	0.55	12.0	13.77	14.00	13.82 ± 0.17	1.23	8.9
4	66	9	5.57	6.83	5.72 ± 0.10	0.83	14.5	15.45	15.86	15.50 ± 0.10	0.81	5.3
5	39	5	5.17	5.24	5.17 ± 0.08	0.48	9.2	13.85	14.19	13.89 ± 0.14	0.88	6.3
6	48 <sup>5</sup>	0	4.71		4.71 ± 0.10	0.67	14.3	13.25		13.25 ± 0.13	0.91	6.8
7(1)	36	0	4.98		4.98 ± 0.07	0.42	8.4	14.37		14.37 ± 0.14	0.89	6.2
7(2)	41	25	5.55	5.72	5.65 ± 0.08	0.54	9.5	15.17	15.46	15.34 ± 0.13	0.82	5.4
8	38	12	5.19	7.10	5.84 ± 0.19	1.17	20.1	14.23	16.11	14.82 ± 0.25	1.17	10.6
9	40	22	6.64	7.29	6.98 ± 0.15	0.93	13.3	15.54	16.59	15.96 ± 0.18	1.12	7.1
10	40	29	4.90	6.23	5.76 ± 0.18	1.17	20.3	13.64	15.56	15.04 ± 0.25	1.59	10.5

<sup>1</sup> Two lactations are shown for cow number 7.

<sup>2</sup> Standard deviation.

<sup>3</sup> Coefficient of variation.

<sup>4</sup> Standard error of mean.

<sup>5</sup> Milk criticized as salty during entire lactation.

which presents statistical constants for the mean fat content of the milk of a complete lactation for each of the cows. The mean fat content varied from 4.71 per cent to 6.98 per cent, with coefficients of variation of from 8.4 to 20.1 per cent.

The general tendency for the fat content of milk to decrease during the first weeks of lactation and later to increase is shown in Figure 1; the time of onset of the increase varied with the individual animal. The milk of animal 7 was exceptional in that in both the lactations shown, the fat increased during the first third of the lactation period then decreased slightly as lactation advanced.

Weekly changes in the mean fat content of normal milk of the herd throughout lactation is shown in Figure 2, graph 1. This curve was estab-

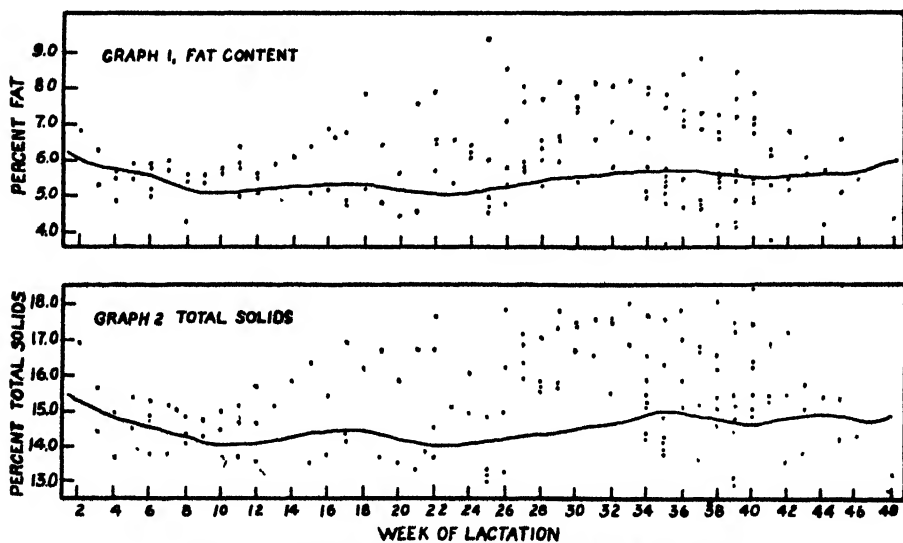


FIG. 2. Fat and total solids content of Jersey milk.

Graph 1

— Mean fat content of all normal samples for each week of the lactation period.

● Fat content of rancid samples.

Graph 2

— Mean total solids content of all normal samples for each week of the lactation period.

● Total solids content of rancid samples.

lished by the analysis of 672 individual samples obtained from 20 cows. The general trend of the fat content of milk was marked by a sharp decrease from 6.2 per cent to 5.0 per cent during the first ten weeks of lactation followed by a slow and irregular increase until a fat content of 5.77 per cent was reached in the 48th week.

The above data have been summarized in Table 2 showing the mean fat content of all normal samples for each of 12 four-week periods. The coeffi-

TABLE 2  
*Fat and total solids content of normal<sup>1</sup> Jersey milk in relation to the period of lactation*

Lactation period	Milk samples	Fat content			Total solids content		
		Mean	S. D. <sup>2</sup>	C. V. <sup>3</sup>	Mean	S. D. <sup>2</sup>	C. V. <sup>3</sup>
weeks	number	per cent	per cent	per cent	per cent	per cent	per cent
1-4	55	5.72 ± 0.17 <sup>4</sup>	1.20	21.9	14.85 ± 0.18	1.36	9.1
5-8	66	5.29 ± 0.12	0.99	18.7	14.43 ± 0.14	1.15	8.0
9-12	57	5.00 ± 0.11	0.77	15.3	14.03 ± 0.12	0.93	6.6
13-16	60	5.20 ± 0.11	0.86	16.4	14.24 ± 0.14	1.10	7.8
17-20	58	5.15 ± 0.11	0.87	16.8	14.27 ± 0.17	1.26	8.8
21-24	61	4.99 ± 0.08	0.67	13.3	14.05 ± 0.13	1.01	7.2
25-28	64	5.20 ± 0.10	0.82	15.8	14.31 ± 0.14	1.08	7.6
29-32	66	5.32 ± 0.11	0.86	16.1	14.50 ± 0.15	1.17	8.1
33-36	54	5.54 ± 0.16	1.13	20.3	14.91 ± 0.20	1.44	9.6
37-40	39	5.36 ± 0.15	0.90	16.9	14.51 ± 0.20	1.25	8.6
41-44	34	5.45 ± 0.13	0.73	13.3	14.71 ± 0.16	0.96	6.5
45-48	20	5.55 ± 0.11	0.48	8.7	14.82 ± 0.23	1.01	6.8

<sup>1</sup> No rancid samples are included.

<sup>2</sup> Standard deviation of mean.

<sup>3</sup> Coefficient of variation of mean.

<sup>4</sup> Standard error of mean.

coefficients of variation obtained for the fat content of milk from different cows in the same period of lactation were high, ranging, with one exception, from 13.3 per cent to 21.9 per cent, and were somewhat greater than those found for the fat content of the milk of individual animals during a lactation. The average fat content of 672 normal samples was  $5.35 \pm 0.035$  per cent, with a standard deviation of  $\pm 0.912$  per cent, and a coefficient of variation of 13.57 per cent.

The decrease in fat content during the first three months of lactation is in agreement with the findings of Van Slyke (7), Eckles (2), Ragsdale (5), and Becker (1), although the drop observed above is somewhat greater than that found by these investigators. The subsequent increase reported by these workers is more regular and slightly greater than was found in the herd under observation. Grady (3), Ragsdale (5), and Becker (1), reported an average fat content of 4.98, 4.98 and 4.605 per cent, respectively, in Jersey milk for the first month of lactation and final values of 5.75, 5.73 and 5.55 per cent, as compared with 5.72 and 5.55 per cent, the initial and final values reported here.

#### *Fat Content of Rancid Samples*

A comparison of the fat content of normal milk with that of rancid milk produced during a lactation may be made from the individual graphs in Figure 1 and from the summarized data in Table 1. From these it is evident that the average fat content of rancid samples exceeded that of normal samples from the same animal and that the average fat content of all samples taken during a lactation was higher for animals 7 (2), 8, 9 and 10, frequently producing rancid milk, than for animals 1, 6 and 7 (1), which produced no rancid samples. The average for the former group ranged from 5.65 per cent to 6.98 per cent, that of the latter from 4.71 per cent to 4.98 per cent. It is also of interest to note that during lactation 1 of animal 7, when no rancid milk was produced, the average fat content of the milk was 4.98 per cent, whereas in the succeeding lactation when 61 per cent of the samples analyzed were rancid, the average fat content was 5.65 per cent.

In Figure 2, graph 1, one may compare the fat content of rancid samples with the average fat content of normal milk in the same week of lactation. The tendency is obviously toward a higher fat content in the rancid samples, although many of the values fall within the limits of normal variation as set down in Table 2.

Table 3 shows the average fat content of all normal samples and of rancid samples grouped according to their degree of rancidity and without regard to the period of lactation in which they were produced. The average fat content of the three groups of samples designated as "rancid," "slightly" and "doubtfully rancid" were 6.17, 6.02 and 6.07 per cent, respectively, and were appreciably higher than the mean fat content of 5.35 per cent for all

TABLE 3  
*Fat and total solids content of normal and rancid Jersey milk*

Description of samples	Milk samples	Fat content		Total solids content	
		Mean	Standard deviation	Mean	Standard deviation
	<i>number</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Very rancid . . . . .	5	5.31	0.34	14.96	0.34
Rancid . . . . .	33	6.17	0.99	15.79	1.25
Slightly rancid . . . . .	64	6.02	1.40	15.28	1.79
Very slightly rancid . . . . .	18	5.21	0.53	14.69	1.48
Doubtfully rancid <sup>1</sup> . . . . .	47	6.07	1.08	15.45	1.38
Rancid (total) <sup>2</sup> . . . . .	167	5.96 ± 0.09	1.19	15.35 ± 0.12	1.52
Normal (total) . . . . .	672	5.35 ± 0.04 <sup>2</sup>	0.91	14.53 ± 0.02 <sup>2</sup>	0.53

<sup>1</sup> Criticized as rancid by less than half the judges.

<sup>2</sup> Standard error of mean.

<sup>3</sup> Total samples scored as having some degree of rancidity.

normal samples. Peculiarly, the few samples described as "very" rancid had a fat content practically the same as that of normal milk.

The average fat content of 167 rancid samples was 5.96 per cent as compared to 5.35 per cent the average of 672 normal samples. The difference between the two values is 0.61 per cent which is 6.2 times the standard error of the difference,  $\pm 0.0986$  per cent, and is therefore significant.

#### *Total Solids Content of Normal Jersey Milk*

The total solids content of milk paralleled that of the fat content, showing similar fluctuations from week to week. Weekly variation in the total solids content of the milk of individual cows are shown by graphs in Figure 3. The degree of the variations is indicated in Table 1 by statistical constants for the mean total solids content of the milk of the individual cows for an entire lactation. The mean total solids content for the ten animals represented varied from 13.25 per cent to 15.96 per cent, with coefficients of variation ranging from 4.79 to 10.54 per cent, with an average coefficient of 7.29 per cent.

Weekly changes in the mean total solids content of the milk of the herd throughout lactation are shown by graph 2 in Figure 2. The trend of total solids content throughout lactation was the same as that observed in the fat content. During the first 10 weeks the total solids decreased from 14.85 per cent to 14.03 per cent, after which it took an upward trend, interrupted at times by slight drops, reaching a final value of 14.82 per cent in the 48th week of lactation.

The statistical significance of the above data is shown in Table 2 which presents constants for the mean total solids content of all normal samples for each of the 12 four-week periods. Variation in the total solids content of milk of different animals in the same period of lactation was the same as that shown by the individual animal in the course of a lactation. The mean total

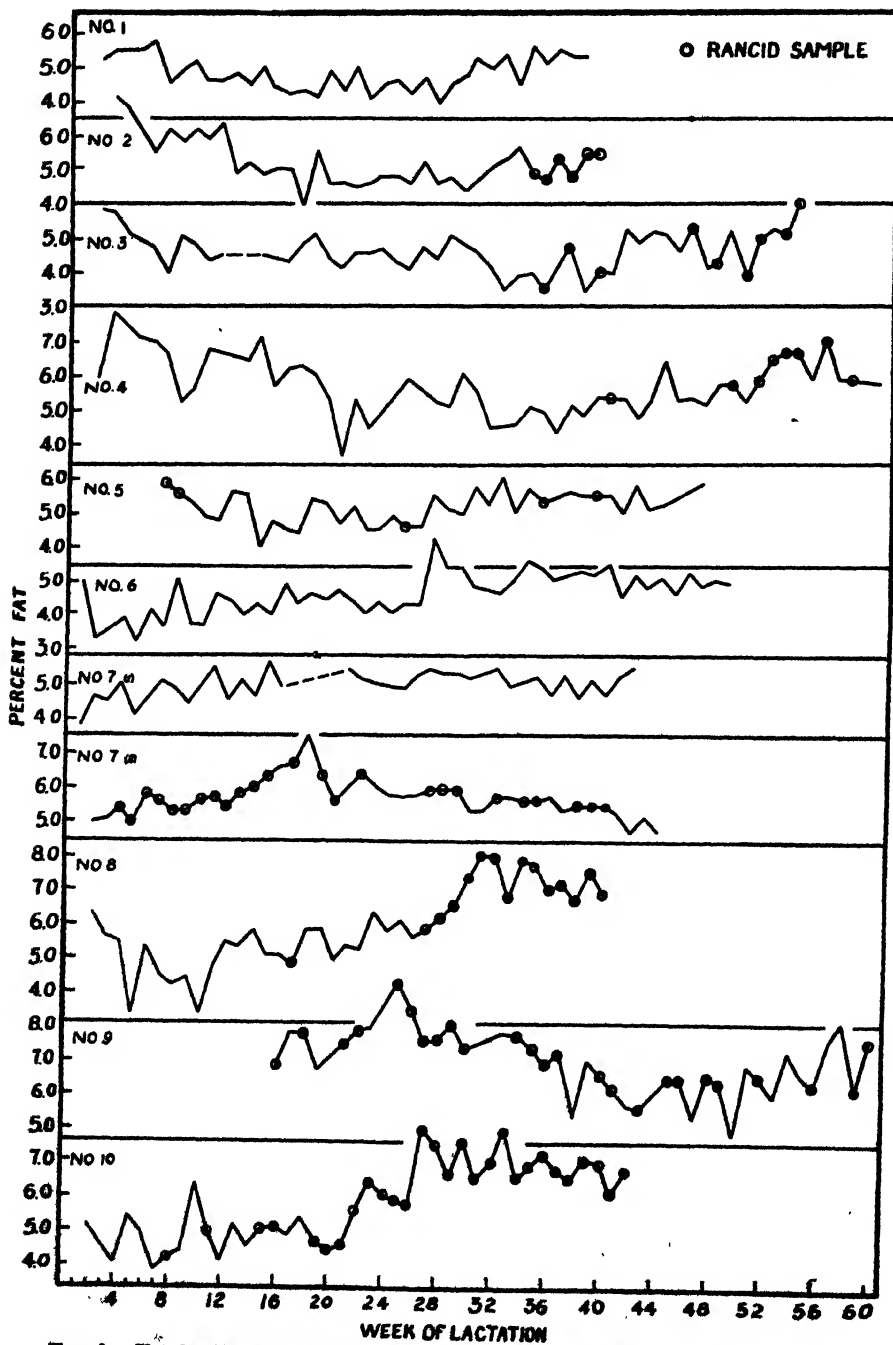


FIG. 3. Total solids content of milk samples taken weekly from each of ten Jersey cows during a complete lactation. Rancid samples are indicated by circles.

solids content of 677 normal samples irrespective of the period of lactation was  $14.53 \pm 0.0205$  per cent, with standard deviation of  $\pm 0.534$  per cent and a 3.7 per cent coefficient of variation.

#### *Total Solids Content of Rancid Milk*

In Figure 3 the total solids content of rancid samples may be compared with that of non-rancid samples of the same lactation. As is shown in Table 1, the mean total solids content of rancid samples exceeded that of normal samples produced in a given lactation and the average total solids of all samples produced during a lactation was higher for animals that frequently produced rancid samples than for those that did not. The milk of animals 7, 8, 9 and 10, had an average total solids content of 15.29 per cent; that of animals 1, 6, and 7 (1) an average of 13.89 per cent. The average total solids for the milk of animal 7 during lactation 1 when no rancid samples were produced was 14.37 per cent, whereas in the succeeding lactation during which many rancid samples were produced, it rose to 15.34 per cent.

From Graph 2, Figure 2, it is evident that the total solids content of rancid samples was usually higher than the mean total solids content of normal samples produced during the same week of lactation. The average total solids content of 163 rancid samples was  $15.35 \pm 0.12$  per cent; that of 672 normal samples  $14.53 \pm 0.02$  per cent. The difference between the two values, 0.82 per cent, is 6.79 times the standard error of the difference, 0.1207 per cent, and is therefore significant.

#### *Protein Content of Normal Jersey Milk*

Determinations were made of the protein content of all samples collected over a period of about four months. A total of 290 samples were analyzed for total protein and of these, 77 samples were analyzed for casein and albumin.

Table 4 presents the average protein content of all normal samples for 12 successive four-week periods. The behavior of protein as lactation advanced was characterized by a decrease from 3.6 per cent in the first month to 3.3 per cent in the second month of lactation, followed by an increase lasting until the 7th month when the protein reached a value of 3.8 per cent which was maintained with little change until the 12th month. Twelve normal samples taken during the 16th to 18th periods were found to have an average protein content of 4.34 per cent. Except for the 4th and 5th months, variation in the protein of samples taken in the same period of lactation was small, the coefficients of variation ranging from 3.67 per cent to 9.21 per cent. There was also little variation in protein of samples from the same animal, the coefficients of variation for 14 individuals ranging from 5.6 to 8.3 per cent. The mean protein content of all normal samples was  $3.60 \pm 0.02$  per cent, with a standard deviation of 0.36 per cent and a coefficient of variation of 9.92 per cent.

TABLE 4  
*Protein content of normal<sup>1</sup> Jersey milk in relation to the period of lactation*

Lactation period	Milk samples	Protein content		
		Mean	Standard deviation	Coefficient of variation
<i>weeks</i>	<i>number</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1-4 .....	19	$3.63 \pm 0.07^2$	0.32	8.8
5-8 .....	37	$3.33 \pm 0.04$	0.24	7.3
9-12 .....	35	$3.36 \pm 0.04$	0.23	6.7
13-16 .....	32	$3.54 \pm 0.08$	0.43	12.2
15-20 .....	28	$3.61 \pm 0.08$	0.43	11.9
21-24 .....	23	$3.67 \pm 0.04$	0.17	7.5
25-28 .....	21	$3.83 \pm 0.07$	0.33	8.7
29-32 .....	15	$3.76 \pm 0.04$	0.14	3.8
33-36 .....	9	$3.76 \pm 0.06$	0.18	4.9
37-40 .....	11	$3.83 \pm 0.11$	0.35	9.2
41-44 .....	8	$3.85 \pm 0.07$	0.20	5.1
45-48 .....	6	$3.75 \pm 0.06$	0.14	3.7

<sup>1</sup> No rancid samples are included.

<sup>2</sup> Standard error of mean.

Variations in the protein of milk observed in this study are, in general, in agreement with earlier findings. Nottbohm (4) in a study carried out with one animal observed that protein increased considerably during lactation, a sharp rise occurring after the 36th week. Van Slyke (6) found that the per cent protein dropped from 3.19 per cent in the first month to 2.99 per cent in the second, then began an increase which continued throughout during the entire lactation. The increase became more marked in the tenth and eleventh months. The maximum value observed was 4.04 per cent and was reached in the eleventh month. Eckles and Shaw (2) observed no drop in protein of Jersey milk between the first and second months, but they found that the protein increased after the second month from 3.32 to 4.91 per cent in the last period (56th week). They report an average of 3.64 per cent total protein for the complete lactations of three animals, as compared with 3.60 per cent the average value found for all normal samples in the present study.

#### *Protein Content of Rancid Jersey Milk*

During the period in which the protein content of milk was studied, 46 rancid samples were produced. Twelve rancid samples occurring in the first 16 weeks of lactation had a protein content ranging from 2.71 per cent to 3.91 per cent as compared with the normal range of from 3.33 per cent to 3.54 per cent for the same period. Thirty-four rancid samples occurred after the 28th week of lactation; of these, thirty-two had a higher protein content than did normal milk of the same period, the values ranging from 4.0 per cent to 5.32 per cent. The protein content of normal milk of the same period varied from 3.76 per cent to 3.85 per cent.

The average protein content of all rancid samples was  $4.13 \pm 0.096$  per cent, standard deviation  $\pm 0.6511$  per cent and a coefficient of variation of 15.8 per cent. The standard error of the difference between the mean

TABLE 5  
Total protein, casein and albumin content of normal and rancid Jersey milk

Description of samples	Milk samples	Total protein		Casein		Albumin		Casein	Albumin
		Mean	S. D. <sup>1</sup>	Mean	S. D.	Mean	S. D. <sup>1</sup>		
Normal	number 67	per cent 3.68	per cent 0.559	per cent 2.88	per cent 0.54	per cent 0.35	per cent 0.06	per cent 78.75	per cent 9.41
Rancid	10	4.05	0.359	3.18	0.35	0.39	0.06	78.50	9.53

<sup>1</sup> Standard deviation of mean.

protein content of normal and rancid milk was  $\pm 0.096$  per cent; the difference was 0.53 per cent, which is 5.4 times the standard error of the difference and is therefore significant.

### *Protein Distribution in Normal and Rancid Jersey Milk*

Since the total protein content of rancid milk was found generally to be higher than that of normal milk, it seemed desirable to determine whether the increased protein was due to an increase in one or all of the protein fractions. Determinations were made of the total protein, casein, and albumin content of 67 samples of normal milk and 10 samples of rancid milk. The results of these determinations are shown in Table 5. From these results it is evident that casein and albumin are present in the same proportion in rancid milk as in normal milk. One would conclude, therefore, that the increase in the total protein of the rancid samples is due to an increase in all the protein fractions rather than to an increase in any single one.

### SUMMARY AND CONCLUSIONS

Data have been presented showing the fat, total solids and protein content of the milk of animals of a Jersey herd, all of which received the same ration and were subject to the same environmental conditions. The amounts of these constituents found in milk criticized as rancid have been compared with the amounts present in normal milk produced during the same period of lactation. The data are presented statistically and graphically both for individual animals and for the herd.

In general, rancid milk has a higher content of total solids, fat and protein than does normal milk of the same period of lactation. The increased protein content of rancid milk is attributed to an increase in the amounts of both the casein and lactalbumin fractions. The high content of these constituents appears to be characteristic of all milk produced by those animals whose milk is frequently rancid.

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# INTERRELATIONS OF MILK-FAT, MILK-PROTEIN AND MILK-ENERGY YIELD

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This article grew out of an inquiry by the American Dairy Cattle Club. In the registration of dairy cattle the Club has taken the progressive step of requiring an estimate of milk-protein yield (in addition to the usual estimate of milk and milk-fat yield) of individual cows. The yield estimates are based on monthly tests covering the 3d-307th days of each lactation, or 305-day partial lactation system.

A recent article by Dr. Goodale (1), geneticist for the Club, represents that protein is the most valuable of all the milk components and that it is desirable, by breeding, to increase the ratio of protein yield to yield of other milk solids (comparable to increasing the proportion of high-priced cuts in meat animals).<sup>1</sup> A breeding program along this line requires a practical field test for protein. Pending the development of such a test the question arises as to the possibility of estimating protein yield from the milk and fat yield.

This question may be put in the form of the relation between fat percentage and protein percentage of the milk of individual cows. If we know the 305-day fat percentage for a given cow, how accurately can we estimate her corresponding protein percentage? The relation between fat and protein (as well as other milk components) for 3-day samples has been heretofore reported (2, 3). It is the purpose of this paper to apply the analyses of these 3-day samples to the appropriate milk yields to secure an estimate for individual cows for a partial lactation approximating the above 305-day period, and to present the interrelation between yields of various milk components, particularly milk fat, milk protein and milk energy.

## DATA AND METHODS

The chemical analyses (2, 3) were made on 3-day composite samples of the milk of individual cows in the University of Illinois herd. For the most

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<sup>1</sup> This is a very free version of Goodale's paper, which in fact contains a mixture of legal, economic and biologic points, and unfortunately some misinformation as to milk composition. He states that as we pass from milk of 3 per cent fat to milk of 6 per cent fat the percentage of lactose increases from 4.0 to 4.4 and the percentage of ash increases from .6 to .9. Aside from the gross errors involved in his absolute lactose and ash values it is quite contrary to the well-known principle of osmotic equilibrium in milk secretion that lactose percentage and ash percentage should vary so markedly in the same direction. To maintain a constant osmotic pressure of milk (equal to that of the blood) it is inevitable that variations in lactose percentage and ash percentage will tend to be in opposite directions.

part the samples were taken at 5-week intervals, although there was some variation in this particular. The analysis of each sample included water, fat, protein (total nitrogen  $\times 6.38$ ), ash and lactose (lactose by difference). In all, 2426 samples were analyzed. Milk yield was determined by weighing each milking.

In the present use of these data the analyses of the first 7 to 11 samples of the lactation have been applied each to an appropriate portion of the continuous milk-yield records (the sample being at approximately the center of the milk-yield portion) to estimate the total milk, fat, protein, lactose, ash and water yield for a period approximating the 3rd-307th days of lactation. The actual length of period represented varies somewhat but is here referred to as 305 days. As thus defined, 305-day partial lactations were available for 130 cows, represented by a total of 1519 samples (out of the 2426 referred to above).

From these 305-day yields the average composition of the milk is derived and presented in Table 1, together with the identity of the cow, the number of samples, length of period represented and average milk yield per day for the period. In the table, 7 to 11 samples indicate a single partial lactation; 16 to 21 samples indicate two partial lactations combined as one; 27 to 32 samples indicate three partial lactations combined as one.

Milk energy is estimated from the equation (4),  $E = 93.12f + 53.58p + 39.871 + 49.80a - .356w$ , in which  $E$  is calories of milk energy per kilogram of milk,  $f$  is fat percentage,  $p$  is protein percentage,  $l$  is lactose percentage,  $a$  is ash percentage, and  $w$  is water percentage.<sup>2</sup>

#### RELATION BETWEEN FAT AND PROTEIN

The 305-day fat and protein percentage data of Table 1 are plotted in Figure 1. The correlation between the two is measured by the coefficient of correlation,  $r = .755$ . Protein is related to fat by the linear equation  $p = 2.10 + .346f \pm .085$ , shown by the straight line of Figure 1.

From the standpoint of estimating an unknown 305-day protein percentage from a known 305-day fat percentage by the equation  $p = 2.10 + .346f$  applied individually in a large population of cows, the present data indicate that the estimate would be correct within .085 either plus or minus for one-

<sup>2</sup> Since, by the method of analysis,  $f + p + l + a + w = 100$ , we have the algebraically equivalent equations:

$$\begin{aligned} E &= \pm 0 + 93.120f + 53.580p + 39.8701 + 49.800a - .356w \\ &= +9312.0 \pm 0 \quad f - 39.540p - 53.250l - 43.320a - 93.476w \\ &= +5358.0 + 39.540f \pm 0 \quad p - 13.710l - 3.780a - 53.936w \\ &= +3987.0 + 53.250f + 13.710p \pm 0 \quad l + 9.930a - 40.226w \\ &= +4980.0 + 43.320f + 3.780p - 9.930a \pm 0 \quad a - 50.156w \\ &= - 35.6 + 93.476f + 53.936p + 40.226l + 50.156a \pm 0 \quad w \end{aligned}$$

As a matter of convenience in computation the last one of these equations was used in estimating  $E$ . In applying the equation  $f$ ,  $p$ ,  $l$  and  $a$  were used to three decimals instead of the two reported in Table 1.

TABLE 1

*Composition of 305-day partial-lactation milk yields of 130 individual cows*

Herd No.	Sam- ples	Total period	Milk yield	Fat (f)	Pro- tein (p)	Lac- tose (l)	Ash (a)	Water (w)	Energy per kg. milk
	No.	ds.	kg.	%	%	%	%	%	cal.

14 Ayrshire Cows									
135	18	595	8.4	3.32	3.13	4.48	.67	88.40	657
326	9	288	18.9	3.46	3.45	4.92	.66	87.51	704
304	9	322	10.6	3.94	3.39	4.52	.69	87.46	732
294	17	584	10.5	3.96	3.65	5.07	.68	86.64	770
74	7	245	11.0	4.00	3.09	4.51	.70	87.70	722
342	9	315	8.7	4.00	3.43	5.14	.68	86.75	763
224	18	645	14.2	4.01	3.57	4.81	.69	86.92	760
354	9	308	8.2	4.18	3.30	4.85	.65	87.02	761
320	9	308	6.1	4.19	3.54	4.56	.67	87.04	764
350	9	315	5.5	4.21	3.49	4.64	.66	87.00	766
321	8	273	7.0	4.37	3.73	4.91	.67	86.32	805
323	18	610	7.9	4.38	3.64	4.91	.70	86.37	802
348	7	245	4.8	4.60	3.65	4.65	.71	86.39	814
351	9	308	7.8	4.96	3.67	5.28	.69	85.40	872

17 Brown Swiss Cows									
510	10	280	18.6	3.38	3.33	5.34	.71	87.24	710
479	10	280	15.3	3.44	3.44	5.02	.75	87.35	711
374	21	602	21.5	3.45	3.17	4.94	.71	87.73	692
499	9	252	12.5	3.50	3.61	5.23	.75	86.91	734
426	19	532	14.8	3.51	3.41	5.10	.69	87.29	717
394	29	826	27.8	3.54	3.37	5.11	.72	87.26	719
427	30	833	21.7	3.62	3.05	5.25	.69	87.39	714
435	20	560	24.4	3.63	3.38	5.19	.71	87.09	730
376	10	287	17.2	3.69	3.39	4.86	.70	87.36	722
393	20	574	13.4	3.90	3.21	5.00	.68	87.21	737
395	10	280	16.2	3.90	3.32	5.37	.69	86.72	759
475	21	581	11.6	3.96	3.44	5.09	.73	86.78	762
404	20	553	9.7	3.99	3.46	5.10	.73	86.72	765
439	32	875	16.2	3.99	3.48	4.99	.74	86.80	763
445	20	559	18.7	4.18	3.51	5.14	.73	86.44	788
401	10	280	18.9	4.28	3.55	5.30	.71	86.16	805
480	11	301	14.3	4.35	3.96	5.00	.78	85.91	824

14 Guernsey Cows									
262	9	309	15.9	4.43	3.40	5.01	.71	86.45	798
284	9	308	16.5	4.49	3.42	5.14	.68	86.27	809
335	9	301	18.4	4.59	3.52	4.94	.73	86.22	818
303	18	631	8.2	4.69	3.64	5.03	.75	85.89	839
282	10	330	10.2	4.72	3.57	5.18	.71	85.82	842
300	18	617	8.1	4.82	3.89	4.99	.74	85.56	863
297	9	309	18.1	4.82	3.83	5.07	.74	85.54	863
271	9	315	7.7	4.86	3.97	5.06	.75	85.36	873
272	10	315	9.7	4.89	3.63	4.97	.69	85.82	852
331	9	301	9.4	5.23	4.06	4.89	.76	85.06	907
267	18	644	7.6	5.27	4.25	4.84	.79	84.85	920
301	19	641	7.6	5.31	4.13	4.94	.77	84.85	921
315	9	308	10.1	5.54	3.89	5.06	.73	84.78	932
270	27	926	8.3	5.74	4.16	4.93	.76	84.41	962

TABLE 1—(Continued)

Herd No.	Samples	Total period	Milk yield	Fat (f)	Protein (p)	Lactose (l)	Ash (a)	Water (w)	Energy per kg. milk
	No.	ds.	kg.	%	%	%	%	%	cal.
15 Holstein Cows									
302	9	315	22.7	2.92	3.13	5.10	.65	88.20	644
273	8	308	21.4	3.02	2.99	4.96	.63	88.40	639
254	9	315	28.7	3.04	3.03	4.63	.68	88.62	633
288	9	315	22.7	3.09	3.31	5.11	.66	87.83	671
325	9	295	21.4	3.16	3.46	5.15	.67	87.56	687
263	9	315	29.2	3.19	3.05	4.56	.66	88.54	644
251	9	315	20.5	3.24	2.97	4.82	.67	88.30	654
324	9	302	24.7	3.31	3.25	5.12	.65	87.67	687
200	9	315	17.1	3.36	3.09	4.46	.67	88.42	658
298	19	630	19.0	3.45	3.53	4.86	.70	87.46	708
295	9	315	16.7	3.46	3.38	4.89	.71	87.56	702
322	9	309	18.2	3.50	3.61	5.01	.70	87.18	723
250	8	280	15.5	3.62	3.08	4.92	.67	87.71	700
257	17	624	20.9	3.65	3.29	5.00	.67	87.39	718
296	9	315	13.8	3.67	3.73	4.96	.71	86.93	744
13 Jersey Cows									
333	9	296	18.3	4.37	3.36	4.92	.66	86.69	785
336	9	287	8.6	4.59	3.56	5.19	.69	85.97	829
305	9	312	11.9	4.61	3.59	4.99	.68	86.13	824
279	9	315	10.5	4.68	3.86	5.03	.69	85.74	847
341	9	315	9.6	4.79	3.63	5.32	.70	85.56	857
299	9	349	9.5	4.85	3.77	5.16	.69	85.53	863
313	17	588	11.7	5.00	3.79	4.85	.70	85.66	866
327	19	640	9.1	5.12	3.67	5.09	.68	85.44	880
334	9	308	11.5	5.21	3.88	5.21	.72	84.98	906
317	18	617	9.0	5.32	3.95	5.10	.73	84.90	916
314	9	315	14.0	5.45	4.22	4.89	.74	84.70	935
349	7	238	4.8	5.95	4.12	4.95	.78	84.20	981
347	8	273	4.1	6.00	4.14	5.07	.69	84.10	987
21 Guernsey-Holstein F <sub>1</sub> Cows									
683	9	315	10.4	3.64	3.38	4.68	.73	87.57	712
671	27	938	15.0	3.69	3.42	4.96	.71	87.22	728
665	9	308	17.5	3.81	3.31	4.87	.70	87.31	730
655	9	308	12.8	3.85	3.66	4.78	.77	86.94	752
657	9	301	8.8	3.88	3.45	4.91	.72	87.04	747
663	18	618	16.4	3.93	3.44	4.91	.72	87.00	751
666	9	315	11.0	4.04	3.50	4.77	.76	86.93	760
690	9	301	6.8	4.07	3.66	4.87	.72	86.68	774
680	9	315	10.6	4.13	3.90	5.06	.71	86.20	800
689	9	294	11.5	4.13	3.96	5.28	.73	85.90	814
674	9	308	11.1	4.16	3.82	5.02	.75	86.25	799
673	9	315	16.5	4.19	3.20	5.05	.70	86.86	766
651	9	303	8.5	4.26	3.60	4.68	.71	86.75	781
667	9	308	13.2	4.36	4.06	4.89	.73	85.96	825
668	8	280	10.2	4.37	3.97	4.56	.79	86.31	810
653	7	245	4.9	4.40	4.23	4.53	.78	86.06	825
661	9	301	14.5	4.44	3.45	5.05	.70	86.36	803
688	18	610	10.9	4.75	3.80	5.07	.75	85.63	855
670	19	631	11.2	4.78	3.78	5.02	.73	85.69	854
654	10	815	14.9	4.83	4.01	4.65	.73	85.78	856
659	9	308	11.1	4.93	3.62	5.21	.67	85.57	864

TABLE 1—(Concluded)

Herd No.	Samples	Total period	Milk yield	Fat (f)	Protein (p)	Lactose (l)	Ash (a)	Water (w)	Energy per kg. milk
	No.	ds.	kg.	%	%	%	%	%	cal.
25 Guernsey-Holstein F <sub>2</sub> Cows									
723	19	617	9.6	3.66	3.52	4.91	.69	87.22	728
729	9	308	13.3	3.87	3.18	5.15	.66	87.14	738
739	16	553	9.5	3.88	3.33	5.15	.74	86.90	751
738	10	322	8.5	3.89	3.35	4.81	.70	87.25	736
702	10	316	11.9	3.94	3.39	4.69	.72	87.26	740
713	9	315	8.3	3.98	3.53	4.74	.75	87.00	755
736	8	245	10.9	4.04	3.35	4.97	.72	86.92	759
707	9	301	14.3	4.04	3.57	5.15	.69	86.55	776
732	9	308	8.4	4.21	3.63	4.92	.70	86.54	787
705	9	302	12.6	4.22	3.77	5.21	.70	86.10	807
710	7	238	8.1	4.26	3.60	4.64	.75	86.75	781
714	9	308	10.6	4.29	3.83	5.01	.76	86.11	811
730	9	301	11.4	4.38	3.43	4.99	.73	86.47	796
735	10	299	7.4	4.39	3.58	5.05	.70	86.28	806
711	9	309	10.2	4.42	3.55	4.83	.73	86.47	800
724	18	631	10.4	4.43	3.70	5.01	.71	86.15	815
737	9	308	10.2	4.46	3.26	5.08	.69	86.51	797
715	9	309	7.1	4.53	3.93	4.98	.71	85.85	836
728	9	301	10.6	4.55	3.54	5.07	.70	86.14	819
742	9	315	10.4	4.64	3.34	5.25	.77	86.00	828
725	10	308	11.1	4.64	3.64	5.18	.70	85.84	837
726	9	301	11.6	4.69	3.56	5.05	.71	85.99	833
720	9	296	10.5	4.74	3.68	4.99	.70	85.89	842
716	9	302	6.4	4.83	4.13	4.40	.70	85.94	851
734	8	280	11.1	5.23	3.54	4.96	.74	85.53	881
11 Guernsey-Holstein Back-Cross Cows									
806	9	315	10.3	3.26	3.11	5.05	.64	87.94	673
807	9	322	11.2	3.34	3.19	5.11	.68	87.68	688
804	9	315	12.5	3.41	3.22	5.16	.71	87.50	700
801	18	657	11.7	3.43	3.28	5.06	.70	87.53	701
805	9	315	10.5	3.50	3.30	5.32	.68	87.20	718
648	7	259	13.4	3.77	3.50	4.71	.69	87.33	730
802	7	231	8.9	3.91	3.26	4.92	.68	87.23	738
644	9	322	9.9	4.00	3.56	5.31	.67	86.46	778
649	9	295	12.4	4.07	3.39	5.03	.75	86.76	768
803	9	319	11.1	4.48	3.26	5.02	.65	86.59	794
650	7	238	8.8	4.53	3.95	4.60	.78	86.14	825

half of the individuals, while for the other half it would be incorrect by more than .085 either plus or minus. If milk yield were constant at 10,000 pounds the corresponding "probable error" of estimate of 305-day protein yield would be 8.5 pounds. Considering a 305-day protein yield of 300 or 400 pounds it might seem good enough if the estimate is correct within 8 or 9 pounds for half of the individual cows, and correct within 25 or 30 pounds for any individual in the whole population. However, we do not know that the present lot of 130 cows is representative of dairy cows in general, and use of the equation is not recommended, except as an expedient to obtain a rough idea of protein yield.

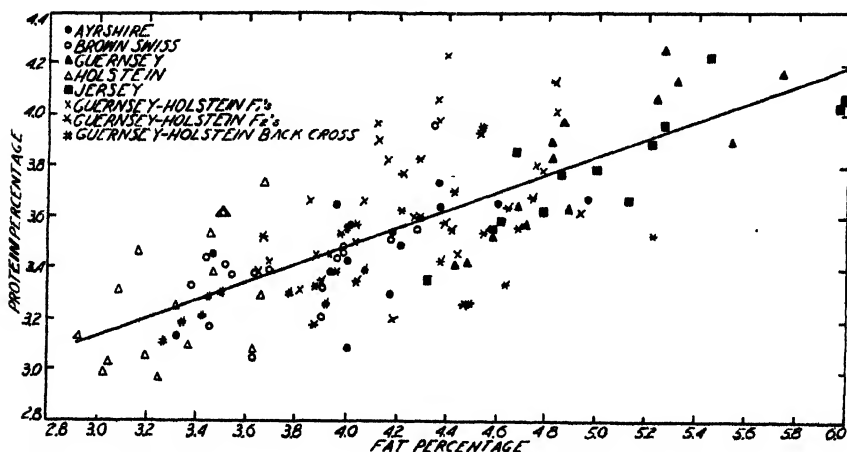


FIG. 1. Per cent protein ( $p$ ) plotted against per cent fat ( $f$ ) for 305-day samples of 130 cows. The correlation is  $r = .755$ , and the regression equation, represented by the straight line,  $p = 2.10 + .346f$ .

In Table 2 the protein-fat percentage equations are given by breed groups, both for the present 305-day samples and the earlier (mixed stages of lactation) 3-day samples. It may be noted from Table 2 that the 305-day protein

TABLE 2  
Protein percentage according to breed and fat percentage

Breed	Protein percentage ( $p$ ) as related to fat percentage ( $f$ )			
	3-day samples		305-day samples	
	Equation	$p^*$	$p^*$	Equation
Ayrshire	$p = 2.061 + .366f$	3.58	3.48	$p = 2.257 + .298f$
Brown Swiss	$p = 1.509 + .523f$	3.53	3.41	$p = 2.091 + .350f$
Guernsey (G)	$p = 1.699 + .447f$	4.02	3.81	$p = .866 + .594f$
Holstein (H)	$p = 1.100 + .653f$	3.42	3.26	$p = 1.514 + .527f$
Jersey	$p = 2.402 + .282f$	3.86	3.81	$p = 1.590 + .438f$
G-H $F_1$ 's			3.68	$p = 2.112 + .371f$
G-H $F_2$ 's			3.56	$p = 2.481 + .249f$
G-H Back Cross			3.36	$p = 2.018 + .355f$
G-H Cross Bred	$p = 1.623 + .499f$	3.80		

\* At mean fat percentage for the breed.

percentage is lower than the corresponding 3-day protein percentage. As between the several breed groups the protein equations show considerable divergence, the greatest contrast being between the Guernsey,  $p = .866 + .594f$ , and the Guernsey-Holstein  $F_2$ 's,  $p = 2.481 + .249f$ . The average of the eight 305-day breed equation constants gives  $p = 1.87 + .398f$  as compared with  $p = 2.10 + .346f$  for the 130 cows as a single group. In the general equation  $p = a + bf$  we are inclined to think it probable that  $b$  has a value of about .4, in spite of the fact that it works out at .346 in the particular case of these 130 cows.

## RELATION BETWEEN FAT AND ENERGY

In Figure 2 milk energy per kilogram of milk is plotted against fat percentage. The correlation between fat percentage and calories for the 305-day samples of the 130 cows is  $r = .9847$ . The relation between these two is much

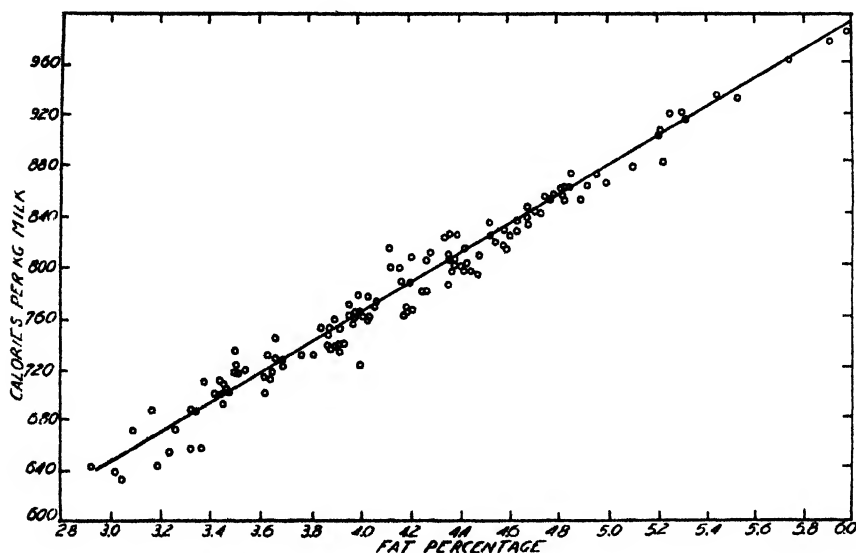


FIG. 2. Calories of milk energy per kilogram of milk ( $E$ ) plotted against fat percentage ( $f$ ) for 305-day samples of 130 cows. The correlation is  $r = .9847$ , and the regression equation, represented by the straight line,  $E = 304.8 + 114.1f$ .

closer than that between fat and protein per unit of milk ( $r = .755$ ) which is natural since fat itself is a direct and dominating contributor to milk energy, while the connection between fat and protein is indirect.

The regression equation is  $E = 304.8 + 114.1f$  or  $E = 114.1 (2.671 + f)$ . This compares with the equation previously found (4) from 1999 3-day samples (which did not include the Brown Swiss data)  $E = 115.33 (2.51 + f)$ . It may be noted that the present 305-day formula agrees very closely with the estimate of milk energy in terms of 4-per cent milk by the formula  $.4 \times \text{milk} + 15 \times \text{fat}$  or 4-per cent milk proportional to  $(2\frac{2}{3} + f)$ . In terms of calories, however, the present equation gives 761 calories per kilogram of 4-per cent milk in comparison with 751 calories by the older (4) equation. It appears therefore that the formula for estimating 305-day energy yield in terms of 4-per cent milk needs no revision, but to convert a kilogram of 4-per cent milk by the  $.4M + 15F$  formula to calories for the 305-day period the factor 761 is indicated, instead of the factor 751 as found from the 3-day samples at mixed stages of lactation.

## RELATION BETWEEN PROTEIN AND ENERGY

In Figure 3 milk energy per kilogram of milk is plotted against protein percentage. The correlation between protein percentage and calories for the 305-day samples of the 130 cows is  $r = .832$ . The relation between these two is not as close as that between fat percentage and calories ( $r = .9847$ ) which

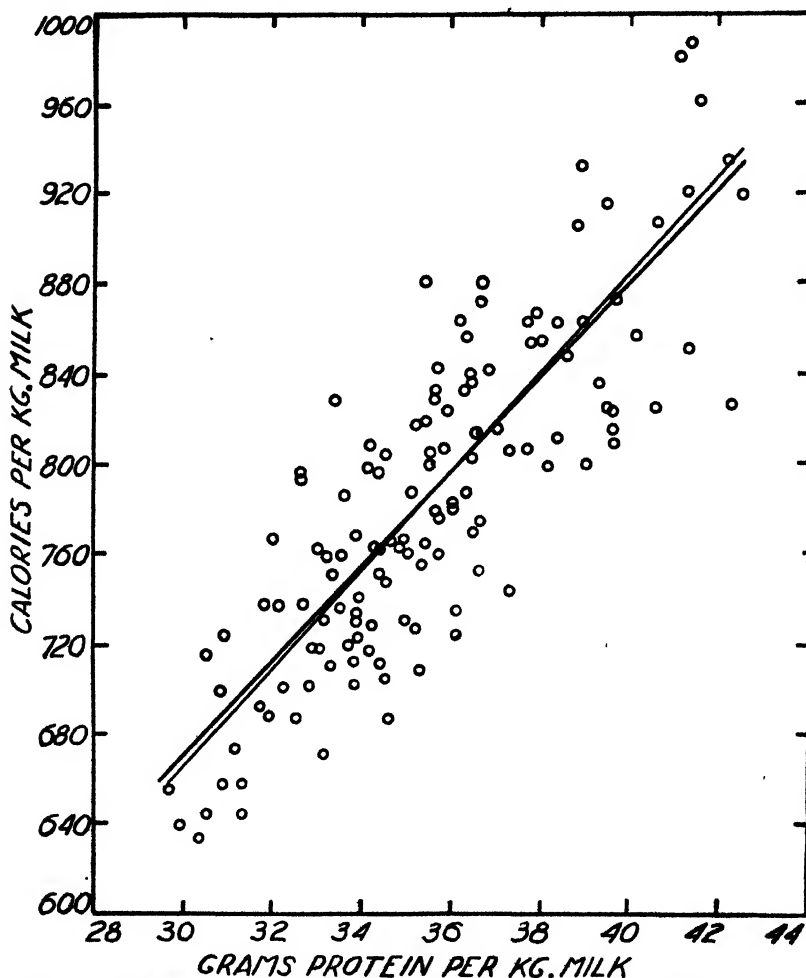


FIG. 3. Calories of milk energy per kilogram of milk (E) plotted against grams protein per kilogram of milk (P) for 305-day samples of 130 cows. The correlation is  $r = .832$ , and the regression equation represented by the heavy straight line,  $E = 37.8 + 21.0P$ . The light straight line represents the equation  $E = 22.6P$ , derived from the means of E and P alone.

may be a reflection of the fact that the protein itself is a smaller contributor to the milk energy than is the fat itself.

The expression of milk energy as a function of the protein (P) of the milk is given in Figure 3 by two equations:  $E = 37.8 + 21.0P$  and  $E = 22.6P$ . The first is the usual least-squares linear regression equation,  $y = a + bx$ ; the second eliminates the  $a$  constant or is simply  $y = bx$  adjusted by least squares, that is,  $b = \text{mean of } x \text{ divided by the mean of } y$ . As may be seen from the plot of Figure 3 there is a very little difference in the accuracy with which the two equations represent the observations. In a gross way fat and energy are more closely related; but in a finer way protein and energy may be more closely related, if one is a simple multiple of the other.

The simple-multiple relation of protein and energy provokes speculation as to the nature of the relationship. If we may say that milk-protein yield is proportional to the nitrogen metabolism of the mammary gland in lactation, and milk-energy yield, to its energy metabolism, then we may advance the thought that the functioning of the gland, as measured by its total energy transformations, is geared to and dependent upon a mechanism of protein growth. This conception fits into the old (discredited) theory that milk formation is accomplished by a process of cell multiplication and disintegration. It may more reasonably be taken to mean that the energy transformations of the milk secreting cell are dependent upon a mechanism of protein elaboration. (In this connection compare the work of Brody, Procter and Ashworth (5) showing that the "basal" energy transformations of various species of animals are proportional to their "basal" nitrogen metabolism.)

By the equation, energy metabolism of lactation = nitrogen metabolism of lactation  $\times$  constant, we reach the conclusion that without nitrogen metabolism of the mammary gland lactation ceases. The milk of all mammals contains protein, so there is no particular instance of contradiction of this conclusion in nature. On the other hand we do have some particular cases (*e.g.*, the mare) in which the milk is nearly fat free, yet lactation proceeds undisturbed and presumably in accordance with the same protein-energy relation. Taking this fat-free milk as origin we may regard fat-rich milk as the product of the original fat-free mechanism plus an additional one (or acceleration of an original weak one) in which we have a simple multiple relation between fat, protein and energy. Again, we may say that a protein mechanism underlies the energy transformations that result in the formation of milk fat.

The milk-protein yield of dairy cows thus takes on a special significance. Furthermore, if we accept the generalization that, as between individual hard-working cows, milk-energy yield tends to be independent of milk composition, then we may deduce the generalization that milk-protein yield tends to be independent of milk composition. Commercially fat yield has been forced on our attention. Biologically it seems that protein yield is more deserving of attention than is fat yield. After all, however, we have the fortunate circumstance that the total work of lactation can be accurately

estimated empirically from the common determinations of milk and fat dictated by commercial necessity.

#### PROTEIN/CALORIE RATIO IN RELATION TO FAT PERCENTAGE

While the foregoing section has placed emphasis on a constant protein/calorie ratio this constancy is only approximate. On the basis of the 3-day samples the equations relating protein per calorie to fat percentage have been reported (4) and are here repeated in Table 3 together with the

TABLE 3  
*Protein calorie ratio according to breed and fat percentage (f)*

Breed	P' = Milligrams of protein per calorie of milk energy			
	3-day samples		305-day samples	
	Equation	P'*	P'*	Equation
Ayrshire .....	$P' = 56.29 - 2.32f$	46.7	45.7	$P' = 58.92 - 3.22f$
Brown Swiss .....			45.9	$P' = 54.77 - 2.34f$
Guernsey (G) .....	$P' = 48.89 - .83f$	44.6	43.7	$P' = 40.47 + .66f$
Holstein (H) .....	$P' = 46.49 + .46f$	48.1	47.9	$P' = 51.44 - 1.08f$
Jersey .....	$P' = 55.59 - 2.32f$	43.6	43.1	$P' = 47.01 - .78f$
G-H F <sub>1</sub> 's .....			46.5	$P' = 55.35 - 2.09f$
G-H F <sub>2</sub> 's .....			44.7	$P' = 57.23 - 2.90f$
G-H Back Cross .....			46.3	$P' = 54.62 - 2.19f$
G-H Cross Bred .....	$P' = 49.68 - .61f$	47.0		

\* At mean fat percentage for the breed.

corresponding equations for the present 305-day samples. In general as fat percentage increases protein per calorie tends to decrease slightly. In the 3-day samples the Holstein breed seemed to be an exception to the general rule. It is therefore of interest to note that in the 305-day samples the exception disappears, and it seems safe to say that for all breeds there is a slight tendency for the amount of protein per calorie to decrease with increase of fat percentage.

#### BREEDING TO ALTER THE PROPORTION OF PROTEIN

As above noted one object of the American Dairy Cattle Club is to promote the breeding of cows in which the milk protein constitutes a larger proportion of the total food value of the milk. Taking energy as a measure of the total food value of the milk, the object is to increase the protein/calorie ratio. From what has been said this object appears difficult, and perhaps in conflict with the principles of the life processes involved in milk secretion. Still, in Figure 3 it is seen that at a given value of calories per kilogram of milk there is a considerable range in the amount of protein per kilogram of milk. That is, the protein/calorie ratio varies to a certain extent as between the individual cows represented in the present 305-day records.

Of the present records 32 cows have two lactations represented. The correlation between the first and second lactations with respect to the protein/calorie ratio for these 32 cows is  $r = .28 \pm .11$ . A part of that correlation is associated with the fat percentage and if fat percentage is held

constant the partial correlation reduces to  $.18 \pm .11$ . If individual differences between dairy cows in the protein/calorie ratio are no more stable than indicated by these low correlations any program of altering the protein/calorie ratio of the milk by selective breeding appears rather hopeless.

#### SUMMARY AND CONCLUSIONS

The data examined consist of the 305-day partial lactation yields of 130 cows with respect to milk fat, milk protein and milk energy. The yields were determined by continuous milk weights and complete chemical analysis of 3-day samples at 5-week intervals.

Where only the milk yield and fat yield are known milk energy yield may be estimated more accurately ( $r = .985$ ) than can protein yield ( $r = .755$ ). The accuracy of estimate of energy yield from milk and protein yield is intermediate ( $r = .832$ ). These correlations are between actual and estimated yields, at a given milk yield.

While the correlation between fat percentage and energy per kilogram of milk is much higher ( $r = .985$ ) than that between protein percentage and energy ( $r = .832$ ) the protein-energy relation is regarded as the more significant biologically. This point of view is based on the fact that energy yield tends to be a simple multiple of protein yield. If there is no elaboration of milk protein there are no lactation energy transformations and there is no milk secretion. On the other hand, elaboration of milk fat may be zero without interrupting milk secretion. The elaboration of milk fat requires the elaboration of milk protein additional to that of fat-free milk secretion. In general, the total (and often enormous) energy transformations of milk secretion depend on and are proportioned to the elaboration of milk protein or nitrogen metabolism of the mammary gland in lactation.

According to the above interpretation it appears futile to try to modify the protein calorie ratio of milk by selective breeding. The protein calorie ratio has a low variability (C.V. = 5) and as between successive lactations of the same cow it shows a low correlation ( $r = .18$ ). Hence, to increase the proportion of food value (calories) present in the milk as protein, by breeding, would be exceedingly difficult.

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# THE pH OF BLUE OR AMERICAN ROQUEFORT CHEESE

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No data have been found in the literature relative to the pH of Blue or American Roquefort cheese. The only information, known by the authors prior to the data secured in this work concerning the pH of Blue cheese, was furnished by Hall (1). According to Hall's data, the pH of Blue cheese shortly after manufacture was 4.55. The pH dropped to a minimum at about pH 4.45 at the end of about 15 days. During the next 15 days there was a rapid decrease in the hydrogen ion concentration of the cheese to pH 4.70. The acidity decreased slowly but quite uniformly as the cheese aged. When the experiment was terminated at 290 days, the cheese was at pH 5.45.

In the course of other experimental work (2) the authors have determined the pH values on cheese from 60 different lots at intervals during the ripening period. The determinations were made daily for 16 days on the cheese from one group of 12 lots, then at intervals of 3 or 4 days until the 29th day, then weekly until the cheese were wrapped in foil on the 99th day, then again at 180 and 270 days. This group is identified as trial 4. The pH determinations were made on the cheese from the other lots at approximately 4, 40, 70, 100, 180 and 270 days.

The mean pH values for the 12 lots of cheese in trial 4 at the various periods are shown in chart 1. Also shown are the mean pH values for the

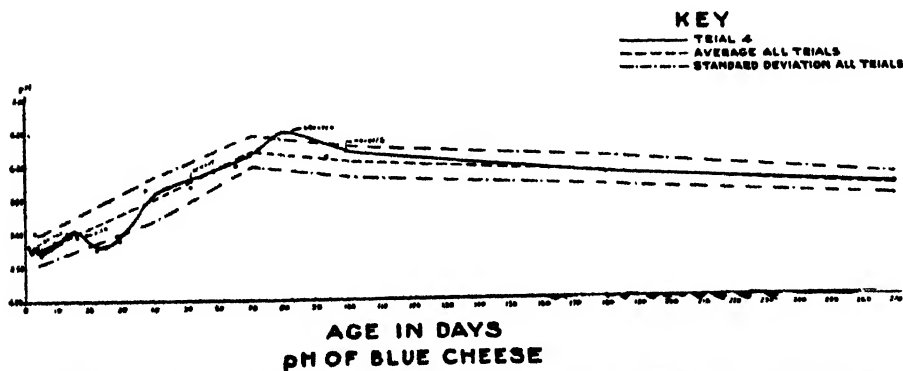


CHART 1. Curve showing the changes in the pH of blue cheese with advancing age.

cheese in all trials at the periods for which data are available. To indicate the variability of the data, the standard deviations of the pH values for the cheese in all the trials are shown.

The hydrogen ion concentration of the cheese appeared to reach a maximum at about pH 4.70 after about 24 hours. After salting, the pH increased

\* The data in this paper are from a thesis presented by J. Spencer George in partial fulfillment of the requirements for the degree of M.S., University of Minnesota, Journal Series Paper 1579. Minnesota Agricultural Experiment Station.

rather rapidly until the cheese were pierced to admit air. The data from trial 4 indicate that following piercing, the pH dropped from about 5.0 to about 4.8, but after a few days, the acidity again decreased. The cheese in trial 4 were pierced on the 19th day at which time the mean pH was 5.01. On the 22nd day the mean pH was 4.77. This difference is statistically very significant, as the value of  $F$  (ratio of the greater to the lesser mean square) is 37.4. According to Snedecor (3) if the value of  $F$  for this number of observations is as great as 7.88 there is only one chance in one hundred that there is no real difference in the data. Every one of the twelve lots of cheese in trial 4 had a lower pH on the 22nd day than on the 19th day.

On the 79th day the mean pH of the cheese in trial 4 was 6.72. This was the maximum pH reached. A gradual re-acidification of the cheese occurred following this period. At 180 days the mean pH was 5.89, and at 270 days 5.72.

The mean pH values for the 60 lots of cheese in all trials followed much the same trend as the cheese in trial 4. The drop in pH following piercing is not evident in the data for all trials as the pH values were not determined between the 4th and the 40th days. The maximum pH recorded for the cheese in all trials was on the 70th day; however, the actual maximum was probably reached as in trial 4, sometime between the 70th and the 100th days.

The cheese in all trials showed the same trend in gradual re-acidification with advancing age as the cheese in trial 4. The mean pH on the 70th day was 6.24, on the 100th day 6.07, on the 180th day 5.91 and on the 270th day 5.69. The value of  $F$  in comparing the pH values of the cheese on the 70th day and on the 270th day is 9.68. A value of  $F$  as great as 6.84 for this number of observations indicates that there is less than one chance in one hundred that there is no real difference in the data.

#### SUMMARY AND CONCLUSIONS

1. The acidity of Blue cheese in these trials reached a maximum at about pH 4.7 within 24 hours after manufacture.
2. With the exception of a temporary increase in acidity following piercing, the acidity decreased gradually to about pH 6.5 at about the end of the third month.
3. After about the third month, the acidity of the cheese increased gradually to about pH 5.7 at the end of the ninth month when the experiment was terminated.

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# A PLANT STUDY OF DAMAGED AND DEFECTIVE MILK BOTTLES

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In recent years much consideration has been given to the economic and practical importance of broken, damaged, and defective milk bottles. Kouwenhoven (1) reported the results of efforts to improve the resistance of bottles to chipping and recommended a group of tests for bottle quality control. Clement, Bain, and Grant (2) studied bottle breakage from the standpoint of plant design. They found that many bottles were discarded because of chipping and that wide and rapid changes in temperature were important factors in breakage. They concluded that bottle breakage in the plants studied depended to a large degree on the plant arrangement and the equipment used. Jones (3) and Antwerpen, Trebler, and Shrader (4) reported on the factors influencing bottle scratching and etching. Mohr (5) discussed the problem of bottle breakage and bottle loss. Dey (6) reported a plant study of bottle damage in which he concluded that defective new bottles, the washing operation, and improper handling in cases and crates were the chief causes of bottle damage.

These studies have been largely concerned with the effect of plant equipment and handling on bottle breakage. The present study was made to determine the character and source of the defects which render milk bottles unfit for use; the occurrence of defective bottles in the daily cycle of usage; and the relation of retail-wholesale sales distribution to the life of the bottles. Two large milk plants using bottles of different manufacture were selected for the study. About 50% of the quarts used in Plant B were Cream-top style. Plant A used the regular or "straight neck" style exclusively.

## I. REJECTED DEFECTIVE BOTTLES

At each point in the plants where defective or broken bottles were picked out, cases were substituted for the cullet cans and the bottles were saved for examination. Paper bags were provided for bottles broken into several pieces. The locations of the bottle collection stations were as follows: (1) return-bottle receiving room, (2) washer inlet, (3) washer outlet, (4) bottle fillers, (5) case filling table, and (6) cold storage room. At the end of the day's operation the rejected bottles were moved to a convenient well-lighted place for a careful examination. A record was made for each bottle showing the bottle manufacturer's name, the year of manufacture, the type of bottle, and the defect which resulted in the rejection of the bottle. A careful study of the bottles resulted in a classification of defects as follows:

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### 1. *Impact shock*

Impact or mechanical shock damage is identified by the presence or remnant of a scar or shatter spot on the bottle at the point of impact. When the bottle is badly dismembered, as is frequently the case in this type of breakage, the edges of the glass fragments have a rippled or streaked surface which serves further to identify the type of breakage. Spalls and chips are a special form of impact shock breakage resulting from a sharp angular or glancing blow. The plane of fracture is across the surface and not through to the inner surface as in regular impact shock breakage. A spall is essentially a chip in which the flake of glass produced is not entirely severed and clings in place. Some spalls extend deep into the surface. Large spalls are commonly known as "butterflies." Chips and spalls are formed most readily on the peaked and rounded regions of bottles such as lips, shoulders, bottom rims, and bottom ridges. (See Fig. 1.)

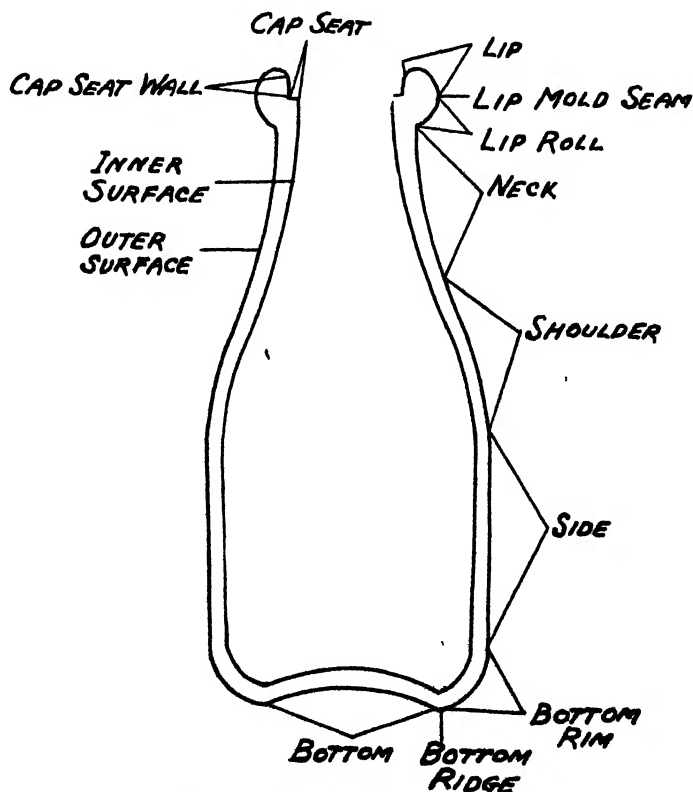


FIG. 1. Milk bottle nomenclature.

### 2. *Thermal shock*

Thermal shock cracks are non-ragged and curve smoothly. The edges of the glass severed by the crack are free from the ripples or grainy appearance

of impact cleavage. The ends of thermal shock cracks usually fade to a point of invisibility and under some conditions the cracks may appear to shorten and lengthen as the bottles are warmed and cooled. Thermal shock cracks do not always extend from outer to inner surface but may be in either surface and extend only partly through the glass. Fine, almost invisible, thermal shock cracks may require a sudden cooling effect such as cold milk at the filler to open them. Inhomogeneity of the glass composition or abnormal tensions on the bottle surface as a result of improper annealing reduces the bottle's resistance to the normal temperature changes in the washer and at the filler. It is these weakest bottles which crack first when the temperatures are not accurately controlled in the washer. Spalls, chips, and manufacturing flaws may act as nuclei for thermal shock cracks.

### 3. Etching

Etching of bottles is accumulative and results mainly from the continual rubbing together of bottles on the conveyors from washers to fillers. This defect is practically absent from bottles in plants where the bottles average less than 25 trips. Etched bottles are rejected because of their bad appearance.

### 4. Manufacturing flaws

Stones are opaque or transparent particles of foreign material in the glass mass. Bottles containing stones are rejected because of their bad appearance. Stones are usually surrounded by areas of improperly distributed compression or tension and act as nuclei for thermal or impact breakage.

Blisters are large air bubbles near the surface of the glass. Blistered bottles are rejected because of bad appearance and also because the thin wall of the blister is easily ruptured leaving sharp edges and a cavity.

Miscellaneous surface flaws are encountered sporadically in new bottles in significant numbers. They result from inefficient inspection at the glass factory. Bottles with such flaws are rejected because of their bad appearance and their susceptibility to breakage. Occasionally lop-sided or otherwise malformed bottles are encountered which present washing, filling, or capping difficulties and must be rejected.

### DISCUSSION OF RESULTS

The damaged and rejected bottles of various sizes picked out at the two plants are classified in Table 1 according to the defect or damage which rendered them unfit for service. It will be seen that spalls and chips occurred most frequently around the lips and the bottom rims of bottles. Bottom ridges were chipped in the small bottles of Plant B largely because of faulty bottle design. In Plant A most of the impact cracking and breakage of the quart size was in the lip and neck. This is probably related to the large

TABLE 1  
Classification of defects in rejected bottles

Type of defect:	No. rejected at Plant A (2 days)				No. rejected at Plant B (1 day)				
	Quart	Pint	$\frac{1}{2}$ Pint	Gill	Quart	Quart*	Pint	$\frac{1}{2}$ Pint	Gill
<b>A. Impact shock</b>									
1. Spalled or chipped:									
(a) Lip and/or cap seat	174	10	52	20	97	45	100	82	21
(b) Shoulder	51	3	0	0	11	73	5	3	1
(c) Side wall	10	2	0	0	7	4	0	0	0
(d) Bottom rim	81	15	37	0	29	83	14	6	2
(e) Bottom ridge	0	0	0	0	0	0	6	22	2
2. Cracked or broken:									
(a) Lip and/or neck	212	11	48	71	24	12	15	65	23
(b) Shoulder	23	4	1	0	5	3	1	4	1
(c) Side wall	32	13	27	0	24	74	18	11	0
(d) Bottom rim	46	11	71	1	20	9	14	12	0
(e) Bottom	9	1	0	0	1	0	0	0	0
(f) Completely	114	3	30	9	34	20	21	4	1
<b>B. Thermal shock</b>									
1. Cracked:									
(a) Lip and/or neck	178	18	16	5	9	33	10	27	33
(b) Shoulder	1	0	0	0	0	0	0	0	0
(c) Side	30	1	9	0	7	3	4	5	0
(d) Bottom rim	4	2	0	0	2	2	0	1	0
(e) Bottom	8	1	1	0	0	0	0	0	0
(f) Mfg. flaw	9	1	0	0	0	0	0	0	0
(g) Completely	6	0	6	2	3	0	1	2	2
C. Etched surface	5	0	0	0	3	0	0	1	0
<b>D. Mfg. defects:</b>									
1. Blisters	3	0	1	0	2	2	1	0	0
2. Stones	3	0	0	0	3	6	2	0	0
3. Flaws	18	1	2	0	4	6	3	0	0
<b>Total No. rejected</b>	1,037	97	301	108	285	375	215	245	86
<b>Total No. washed and bottled</b>	240,000	68,000	78,000	10,000	39,500	39,000	49,000	37,500	5,000
<b>Per cent rejected</b>	0.43	0.14	0.39	1.08	0.72	0.96	0.44	0.65	1.72

\* Cream-top style.

number of thermal shock cracks in that region. A fine almost invisible thermal shock crack may break open as the result of an impact which in itself would not be sufficiently forceful to damage a perfect bottle. There were many thermal shock cracked quarts and pints at Plant A and many gills at Plant B. This was undoubtedly related to the quality of the bottles used, since each plant had the same kind of washing machines and practically the same temperatures were used.

The small bottles followed the general trend of breakage as shown by the quart bottles. Most of the impact shock damage was in the lip and bottom rim regions and most of the thermal shock damage was in the lip region. In both plants the gill bottles had the highest total rejection percentage. They were followed by quarts,  $\frac{1}{2}$  pints, and pints. The low rejection percentage of pints is attributed to their short life as indicated by their low numbers of "trips per bottle." (See Table III). The high rejection percentage of the gills can not be readily explained by the data available.

## II. DEFECTIVE BOTTLES IN USE

In order to study the efficiency of the plant bottle inspection at Plant A and to determine if breakage was occurring in the plant, a special sampling was made at the following stations: (1) return-bottle conveyor; (2) at the bottle washer just after the last chlorine water spray; and (3) at the case conveyor entering the refrigerator. One bottle was picked from the same pocket in every sixth quart case at the conveyors, and one bottle was picked from the same place in every sixth row of the sixteen pocket wide quart bottle washer. This sampling provided approximately 1000 bottles representing each station. These bottles were carefully examined and the defects were noted and tabulated.

A summary of the principal defects found in the bottles at the three stations is shown in Table 2. It is interesting to follow the course of the defective bottles in the plant. Bottles defective as a result of impact shock were

TABLE 2  
*Defects in samples of quart bottles in Plant A*

	Empty returned bottles		Bottles in washer		Filled bottles in refrigerator	
	No.	% of sample	No.	% of sample	No.	% of sample
Type of Defects:						
Impact shock	89	9.4	84	9.1	85	8.7
Thermal shock	8	0.8	13	1.5	9	0.9
Mfg. defects	12	1.2	10	1.1	16	1.6
All defects	109	11.4	107	11.7	110	11.2
Size of Sample	954	100.0	910	100.0	981	100.0

reduced in percentage by the rough inspection at the washer inlet. This included principally bottles with broken necks and sides. The inspection at the washer discharge and at the fillers reduced further the impact shock defects. This latter inspection removed some of the bottles with the largest spalls and chips. The thermal shock cracks were increased or opened up by the washing operation and were again reduced by the washer discharge and filler inspection. It appears from these data that impact shock damage occurs largely outside the plant, and thermal shock cracking occurs in the plant. The manufacturing defects seemed to increase as the bottles passed through the plant. This is an inaccuracy resulting from the fact that it is difficult to see some manufacturing defects, notably transparent stones, until the bottle is filled with milk. A significant fact is that most of the so-called "breakage" at various places in the plant does not necessarily occur at the point where the bottle happens to be picked out. One exception is the washer discharge inspection which removes the bottles seriously cracked by thermal shock in the washer.

### III. RETAIL-WHOLESALE SALES DISTRIBUTION

In Table 3 is shown the approximate distribution of bottled products from Plants A and B to retail and wholesale sales in comparison with "trips

TABLE 3  
*Retail-wholesale sales distribution and "trips per bottle"*

	Plant A			Plant B		
	Retail %	Whole-sale %	Trips per bottle	Retail %	Whole-sale %	Trips per bottle
Quarts	92	8	25.7	61	39	12.2
Pints	85	15	9.4	35	65	5.8
Half-pints	57	43*	12.2	8	92*	13.4
Gills	93	7	14.0	38	62	5.4
Weighted Average	87	13	17.2	52	48	8.9

\* Includes schools.

per bottle." The data shown as "trips per bottle" were obtained by dividing the number of bottles of each size bottled per month by the number of new bottles of each size purchased per month. This was averaged for the period of 9 months prior to the examinations. There were no bottle deposits required in either case and therefore much bottle loss resulted from the wholesale distribution. "Trips per bottle" is largely dependent on bottle loss outside the plant. It should be noted that school sales are included in the wholesale group. It is a large proportion of the total half-pint sales. The bottle returns from schools are excellent.

## SUMMARY

A study of the damaged and defective milk bottles and related data in two large bottling plants resulted in the following conclusions:

1. Bottles were rejected because of impact shock breakage and damages, thermal shock cracks, etched surface, and manufacturing flaws.

2. Spalls, chips, and impact breakage in general occurred most frequently around the lips and the bottom rims of bottle. Sharp edges on the bottles contributed materially to chipping and spalling. Thermal shock cracks occurred most frequently in the lip and neck region. A significant amount of thermal shock cracking occurred in the bottle washing operation.

3. The rejection percentages were in the increasing order: pints,  $\frac{1}{2}$  pints, quarts and gills. There was some relation between low rejection and few "trips per bottle." Large percentages of wholesale distribution without bottle deposit were accompanied by few "trips per bottle."

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# A DIAGRAMMATIC METHOD OF PRESENTING THE HISTORY OF REPRODUCTION IN A DAIRY HERD

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For many years agricultural colleges and experiment stations have recommended the improvement of feeding methods, the elimination of low-producing cows, and the proper selection of breeding stock. Although this program has resulted in more efficient dairy production, there is still room for considerable improvement.

Low production is responsible for only a small percentage of the total number of cows culled. Many are disposed of each year for other reasons, among which sterility and shy-breeding probably stand out as highly important factors responsible for the large number of replacements required annually in the average herd.

Many cows of great potential producing ability are slaughtered and their desirable effects on the herds lost because of their failure to reproduce. The search for superior germ plasm will improve the dairy cow population but little unless such excellent animals can reproduce normally over a period of years.

In studying<sup>1</sup> the causes of low efficiency of reproduction, we wondered whether certain types of reproductive failure might be diagnosed directly from a study of the breeding and calving records. In the literature, however, we have been unable to find a satisfactory method of analyzing such data. This paper will therefore present a diagrammatic method of assembling breeding data for analysis.

A comprehensive survey of the breeding efficiency of a dairy herd naturally requires, first, a complete and accurate daily recording of all breedings, calvings, and veterinary examinations on an easily accessible form. Breeders commonly enter such data in a hip-pocket note book, listing the services and calvings in chronological order. A few use a method resembling that shown in figure 1. The card, measuring  $19\frac{1}{2} \times 17\frac{1}{2}$  inches, will take care of a 40-cow herd. The animals are listed by number in order of age—oldest to youngest. Provision having been made for two calvings, the card need not be renewed oftener than once each year. When any cow has calved twice, a new card is made out, and the cows are again listed in the original order. At birth, heifers are numbered and entered immediately in the proper column. Dates of disposal or purchase are also entered, to the right of the cow number.

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<sup>1</sup> The experimental work reported in this paper became cooperative with the United States Bureau of Animal Industry on February 1, 1937.



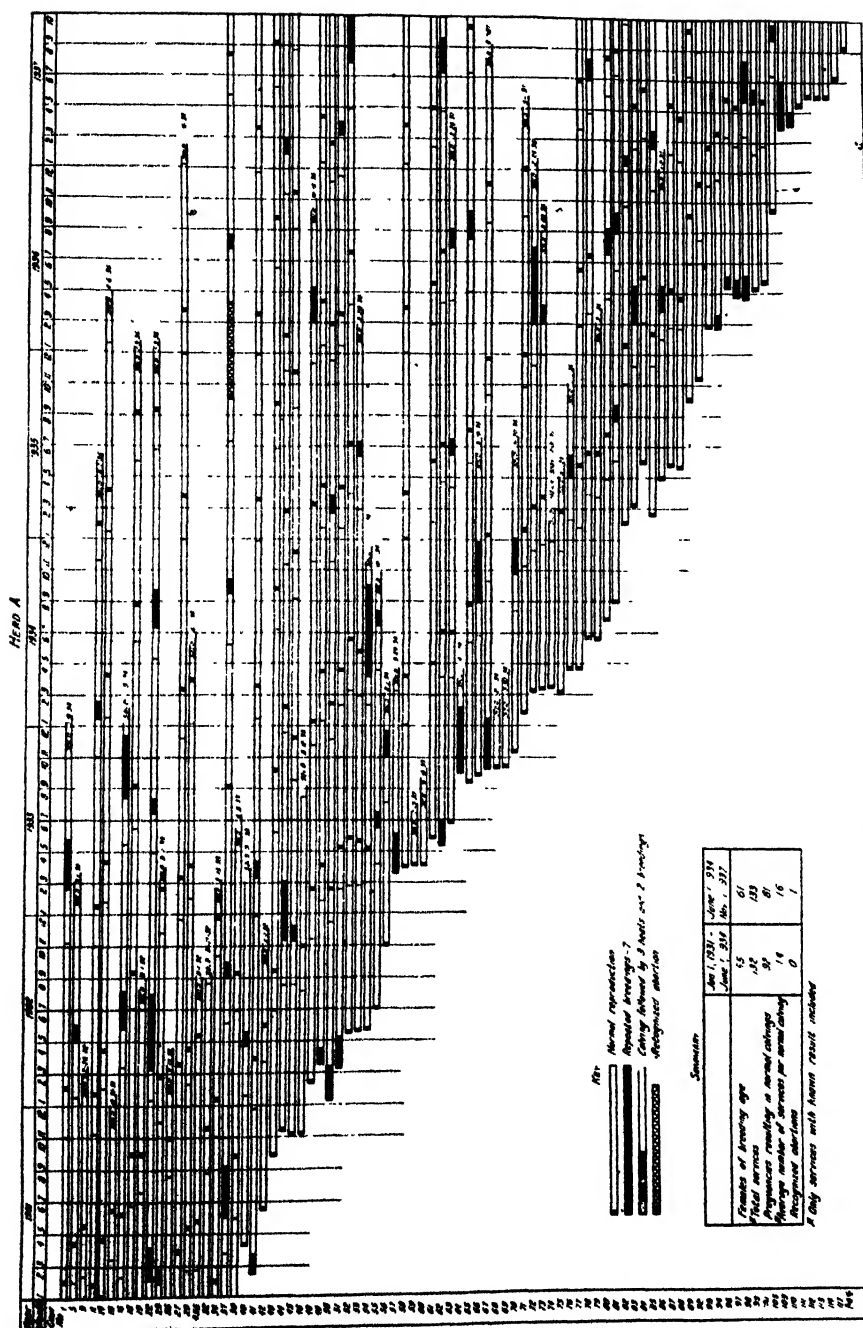


FIG. 2. A diagrammatic presentation of excellent reproduction.

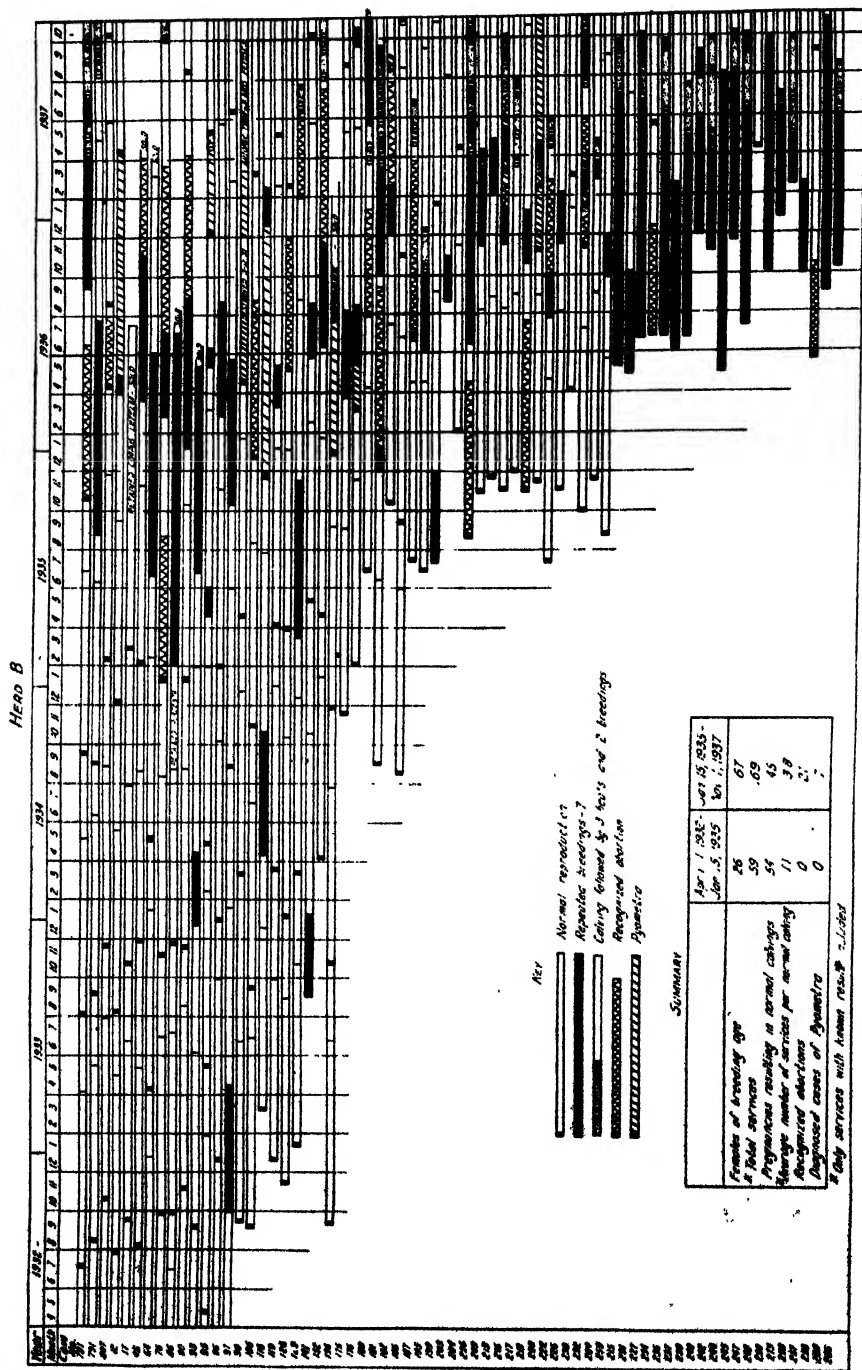


FIG. 3. A diagrammatic representation of efficient reproduction over a period of approximately three years, followed by reproductive failure as a result of Trichomoniasis.

Hence the card serves as an inventory as well as a breeding and calving record. This system has been used in the college herd for fourteen years and by a number of dairymen cooperating with the university in an experimental bull association for ten years. The data when recorded in this manner are readily accessible for analysis.

It is hard to obtain an intelligible picture of the efficiency of reproduction merely through an assemblage of breeding and calving dates. Such data have little meaning when published in tabular form. Figures 2 and 3 attempt to present diagrammatically the histories of reproduction in two California dairy herds.

The former depicts reasonably high efficiency of reproduction, over a period of slightly less than seven years; the latter, normal reproduction for the first 34 months followed by a period of decidedly poor reproduction. The long intervals between breedings, the occurrence of abortions in a herd negative to the agglutination test, and the many cases of pyometra present a typical picture of *Trichomoniasis* as the probable cause, later verified by actual identification<sup>2</sup> of the organism *Trichomonas fetus* (Riedmiller). After this diagnosis, which took place in March of 1937, the chart shows one phase of the disease-elimination measure—the recording of heat periods.

The diagrammatic method of presenting breeding data is flexible and may be adapted to a number of different types of such data. Reports of veterinary examinations are desirable and often essential. Where more than one sire is in use at a time, identification on the chart may be valuable.

A summary of the data recorded in the diagram is not always necessary, since one may obtain a fairly accurate picture of the situation at a glance. Should such a summary be needed, however, the method used will depend upon the viewpoint and the desired result.

#### SUMMARY

A diagrammatic method of showing the history of reproduction in dairy herds is presented. The advantages of this method are as follows:

1. The reproductive history of the entire herd, over a long period, may be seen at a glance.
2. The individual performance of each animal can be easily followed.
3. Frequent recordings of data on the chart permit an early diagnosis of abnormal reproduction.
4. The method greatly facilitates the analysis of the data from various angles.

<sup>2</sup> This verification was made by Dr. H. S. Cameron in the Division of Veterinary Science.



# COMPARISON OF TRYPTONE-GLUCOSE-SKIMMILK AND STANDARD NUTRIENT AGARS AS MEDIA FOR DETERMINING THE BACTERIAL COUNT IN ICE CREAM<sup>1</sup>

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Tryptone-glucose-skimmilk agar has been proposed as a more suitable medium than standard nutrient agar for making total bacterial counts on ice cream and milk. What effect the new medium will have on bacterial counts is of vital interest to the ice cream manufacturer who may be required to meet standards which were adopted on the basis of standard agar counts.

A study of 192 samples of commercial ice cream was made by Babel<sup>2</sup> in which he compared standard agar, standard agar plus sucrose, and tryptone agar. He reported that the tryptone agar gave higher total counts for ice cream than did either of the other two agars. The average logarithmic count for all samples on the standard agar was 43,160 and on the tryptone agar, 108,400, or an increase of 151 per cent. The greatest percentage increase was recorded for the low count samples. Babel noted that 28 of the 192 samples gave lower counts on tryptone agar than on standard agar, 83 gave increases of less than 100 per cent, and 81 showed increases of over 100 per cent.

Another comprehensive comparison of the two media was made by Robertson,<sup>3</sup> in which counts on 412 samples of ice cream were made by five different ice cream companies using the standard agar and tryptone agar at incubation temperatures of 32° C. and 37° C.

The logarithmic average of the bacterial counts obtained on standard agar plates incubated at 32° C. was 137 per cent of the counts obtained at 37° C. The average bacterial counts when tryptone agar was incubated at 37° C. and 32° C. were 116 and 154 per cent, respectively, of the counts made with standard agar. Robertson's study was conducted on ice creams with relatively low bacterial counts.

In the study here recorded, 279 samples of commercial vanilla ice cream were analyzed. The samples were collected by the State Dairy Commissioner of Kansas from licensed manufacturers located throughout the state. Prac-

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<sup>1</sup> Contribution No. 173, Department of Bacteriology and No. 121, Department of Dairy Husbandry.

<sup>2</sup> Babel, F. J. Significance of Laboratory Tests in the Control of Ice Cream. *Ice Cream Trade Journal*, 32, 9, 35. Sept., 1936.

<sup>3</sup> Robertson, A. H. The New Proposed Procedure for Making Ice Cream Plate Counts. Report of Proceedings of 36th Annual Convention, Int. Ass'n of Ice Cream Mfgs., Vol. 2, p. 132. Oct., 1936.

tically all the samples were pint size, factory-filled packages obtained from large wholesale plants, counter freezer operators, and small retail manufacturers. The samples were refrigerated with dry ice, and transported to the laboratory at Kansas State College where the bacterial counts were made. Three plates containing 1/100, 1/1,000, and 1/10,000 dilutions were poured with standard agar and incubated at 37° C. Six plates, two of each dilution, were prepared using tryptone agar, three of which were incubated at 37° C. and three at 32° C. Samples were replated which gave unsatisfactory counts, from the standpoint of number of colonies per plate or discrepancies in counts from the three dilutions.

In Table I the bacterial counts obtained are classified into groups on the basis of the total count on standard agar. The number and per cent of samples falling into each group are indicated. The logarithmic and arithmetic average counts obtained on standard agar have been arbitrarily assigned a value of 100 and counts obtained on tryptone agar at the two incubation temperatures are expressed as percentages of the standard count. The counts are well distributed over a wide range with 57 per cent falling below 100,000 per ml. on standard agar incubated at 37° C.

The logarithmic average counts obtained upon tryptone agar incubated at 37° C. were from 125 to 169 per cent of those obtained on standard agar, the average for all samples being 137 per cent. At the 32° C. incubation temperature the counts varied between 154 and 200 per cent of the counts obtained on standard agar with an average of 192 per cent for all samples (Table I). The arithmetic average counts obtained upon tryptone agar incubated at 37° C. were 142 to 494 per cent of those obtained on standard agar, the average for all samples being 132 per cent. At the 32° incubation temperature the counts varied between 139 and 974 per cent of the counts obtained on standard agar, the average for all samples being 156 per cent (Table I).

As a convenient method of expressing the relationships of the counts, the ratios of the tryptone agar counts to the standard agar counts were calculated by dividing the tryptone agar count by the standard agar count. For example, a ratio of 2.0 would mean that the tryptone agar count was twice that of the standard agar count. These values are presented in Table II. In this table, the relationship of the counts obtained on the different media and at different incubation temperatures is indicated. Sixty-nine per cent of the tryptone agar counts at 37° C. were higher than the corresponding counts on standard agar, whereas at 32° C. 87 per cent of the samples showed higher counts. Comparison of the counts at 32° C. and 37° C. on tryptone agar show that 74 per cent of the samples gave higher counts at the lower temperature. The calculated ratios of tryptone agar counts to the standard agar counts are greater in every instance for the 32° C. temperature than they are at 37° C.

TABLE I  
*Relationship of tryptone-glucose-skimmilk agar counts to standard nutrient agar counts of 279 samples of ice cream*

Range of plate counts	Number of samples	Per cent of samples	Logarithmic averages of standard agar counts (37° C.)	Tryptone agar counts per cent of standard agar counts		Arithmetic averages of standard agar counts (37° C.)	Tryptone agar counts per cent of standard agar counts	
				(37° C.)	(32° C.)		(37° C.)	(32° C.)
Less than 10,000	34	12.2	4,400	125	186	5,000	145	229
10,000-49,999	85	30.5	24,000	148	200	26,500	494	972
50,000-99,999	41	14.7	69,000	145	188	70,700	179	207
100,000-499,999	69	24.7	180,000	133	164	202,000	158	200
500,000-999,999	11	3.9	665,000	135	154	684,500	160	176
1 million to 10 million	25	9.0	2,950,000	169	200	3,652,000	217	276
10 million and over	14	5.0	43,000,000	160	164	100,974,000	142	139
Average all samples	279	100.0	96,500	137	192	5,490,000	132	156

TABLE II  
*Distribution of increases and decreases in count obtained with various media and incubation temperature combinations and the average ratio of counts for each group*

Range of bacteria counts	Number of samples	Tryptone (37° C.) to standard (37° C.)			Tryptone (32° C.) to standard (37° C.)			Tryptone (32° C.) to tryptone (37° C.)			Ratio tryptone (32° C.) standard (37° C.)	Ratio tryptone (37° C.) standard (37° C.)
		Higher	Same	Lower	Higher	Same	Lower	Higher	Same	Lower		
Less than 10,000	34	22	2	10	30	3	1	29	1	4	2.2	1.4
10,000-49,999	85	49	6	30	74	2	9	69	2	14	7.6	3.9
50,000-99,999	41	33	2	6	38	0	3	30	3	8	2.1	2.0
100,000-499,999	69	51	1	17	61	0	8	49	2	18	2.2	1.8
500,000-999,999	11	6	0	5	9	0	2	9	1	1	1.8	1.7
1 million to 10 million	25	22	0	3	22	1	2	16	1	8	4.6	3.3
10 million and over	14	11	0	3	10	0	4	6	1	7	3.7	2.1
Total number of samples	279	194	11	74	244	6	29	208	11	60		

For the entire group of 279 samples the ratios of the tryptone agar counts at 32° C. to the standard agar counts at 37° C. ranged from 0.15 to 325.0 with an arithmetic mean ratio of 4.11, and a median or middle ratio of 1.52. When comparison was made of the ratio of tryptone agar incubated at 32° C. to that incubated at 37° C. it was found that the ratios ranged from 0.16 to 137.0 with an arithmetic mean ratio of 2.46 and a median of 1.25. The mean ratios are somewhat higher than would be expected due to the influence of a few extremely high ratios. The median ratio gives values which compare very closely with the arithmetic average counts. (These values were obtained from a study of all the assembled data and are not shown in the condensed table.)

#### DISCUSSION

It is evident from these data that the tryptone agar gave higher average counts at both 32° and 37° C. incubation temperatures in all the various ranges than did the standard agar at 37° C. This is in agreement with the results reported by other investigators except that the percentage increases are not as great as those obtained in other studies. The incubation temperature was an important factor in determining the count on tryptone agar. The change in incubation temperature from 37° C. to 32° C. produced a greater percentage increase in count than that affected by change in the medium. The logarithmic and arithmetic average counts for the various bacterial ranges were not noticeably different. In some of the comparisons which have been made the percentage increase was greater in the higher bacteria ranges than it was in the lower ranges. In this study no attempt was made to determine what type or types of organisms were responsible for the increased counts obtained on tryptone agar; however, a study is now in progress which may give some information on this point.

The ice cream manufacturer who has had difficulty in meeting present standards will be required to exercise even greater sanitary precautions in the manufacture of his product if the tryptone agar and 32° C. temperature are adopted.

#### SUMMARY

Bacterial counts have been made on 279 samples of commercial ice cream using standard nutrient agar and 37° C. incubation temperature and tryptone-glucose-skimmilk agar incubating the plates at 37° C. and 32° C.

The logarithmic average of the standard agar counts at 37° C. was 96,500 and the counts obtained on tryptone agar at 37° C. and 32° C. incubation temperatures were 137 and 192 per cent respectively of the standard agar count.

The arithmetic average of the standard agar count was 5,490,000 and the counts obtained on tryptone agar at 37° C. and 32° C. were 132 and 156 per cent respectively of the standard agar count.

The mean and median ratios of tryptone agar count at 32° C. to the standard agar count at 37° C. was 4.11 and 1.52 respectively.

The mean ratio of the tryptone agar count at 37° C. to the standard agar count at 37° C. was 2.46 and the median ratio was 1.25.

#### NOTE

Owing to the fact that this manuscript had been completed before the publication of an article entitled, "The Effect of Using Tryptone-Glucose-Skimmilk Agar and 32° C. Incubation on the Bacteria Count of Ice Cream," M. W. Yale and R. C. Hickey, *JOURNAL OF DAIRY SCIENCE*, XX, 12, Dec., 1937, it was not included in the review of literature.

# THE OLD STORY OF TYPE AND PRODUCTION

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The problem of type and production has been of paramount interest to breeders for generations. The importance of desirable conformation was recognized early by the breeders on the Island of Jersey when the first score card or scale of points was drawn up in 1834. It has also been more than fifty years since the first authenticated butter test was supervised by the American Jersey Cattle Club. During all of this time breeders have constantly striven to improve both the conformation and the producing ability of our dairy cattle.

In 1932, the American Jersey Cattle Club established the Jersey Herd Classification program providing for the inspection and rating of Jersey cows and bulls solely on the basis of conformation. The inspections are made by a group of judges approved by the American Jersey Cattle Club and the cattle are given one of six ratings depending upon their conformation, in relation to the scale of points of the Jersey breed. For instance, a cow to be rated as "Excellent" must be an animal which in the opinion of the official judge would score at least ninety or more on the official scale of points. A cow to be classified "Very Good," must in the opinion of the judge be entitled to a score of approximately eighty-five but less than ninety on the official scale of points. The next rating of "Good Plus" is given to animals scoring approximately eighty but less than eighty-five. The rating of "Good" is bestowed upon animals which in the opinion of the inspector would be entitled to a score of approximately seventy-five but less than eighty points. Cows which in the opinion of the judge are entitled to a score of approximately seventy but less than seventy-five points are rated as "Fair" and all animals which in the opinion of the judge would score less than seventy are rated as "Poor."

In applying for Herd Classification, the owner must submit every registered cow which he owns that has ever calved and all bulls fifteen months of age or older. No exceptions are permitted. The program has grown constantly in favor, since its adoption and to January 1st, 1938, a total of 4818 cows and bulls have been officially classified. The number is now large enough to provide some material for statistical analysis and the ratings and the records of these cows and bulls have been studied in the office of the American Jersey Cattle Club for the purpose of throwing further light on the still important question of type and production.

All of the animals classified have been divided into groups depending

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TABLE 1  
Grouping of all animals classified according to age

	Under 2 yrs.	2	3	4	5	6	7	8	9	10	11	12 yrs. and over	Totals
Excellent . . . Cows	0	20	51	37	40	29	28	20	17	21	12	27	302
Excellent . . . Bulls	0	1	8	5	2	6	4	3	3	0	2	4	38
Very Good . . . Cows	0	120	171	142	119	89	65	69	71	53	37	47	983
Very Good . . . Bulls	31	20	19	17	21	6	12	6	3	4	1	7	147
Good Plus . . . Cows	6	284	373	290	241	169	128	85	46	44	27	40	1733
Good Plus . . . Bulls	49	34	17	21	14	9	14	2	8	3	1	2	174
Good . . . Cows	9	261	254	223	139	106	72	60	36	24	15	14	1212
Good . . . Bulls	30	16	4	6	4	6	3	1	1	1	0	2	74
Fair . . . Cows	4	35	39	14	15	10	5	8	3	2	3	4	142
Fair . . . Bulls	0	0	1	0	0	0	0	0	0	0	0	0	1
Poor . . . Cows	0	3	5	1	2	0	0	0	0	0	0	0	11
Poor . . . Bulls	1	0	0	0	0	0	0	0	0	0	0	0	1
Totals . . . Cows	19	723	893	706	556	403	298	242	173	144	94	132	4383
Totals . . . Bulls	111	71	49	49	41	27	33	12	15	8	4	15	435

upon the age at classification and on the ratings given. The results are shown in Table 1.

In examining this table, it will be noticed that there is a decided scarcity of animals in the lower two classification groups. This does not mean that all of the cows of the breed are superior to these lower classifications. However, it is a poor advertisement for any herd to be classified showing a considerable percentage of "Fair" and "Poor" cows. Consequently the usual practice in applying for classification is for the owner to cull the herd in advance and to dispose of the cows of inferior type before the inspection is conducted. As a matter of fact, this stimulant to culling is one of the advantages of the classification program and as a result when the judge arrives at the farm the cows of poor conformation have usually been sold. (It is hoped, to the butcher.)

It is interesting to note that fifty-one per cent of all cows classified as "Excellent" were past six years of age while in the "Good" classification only twenty-seven per cent were six years of age or older and only twenty-five per cent of the "Fair" cows were past six years of age. Apparently, the poorer type cows are soon weeded out of the herds, while the cows of good conformation are retained.

A considerable number of cows which have been classified have also completed official production records either in the Register of Merit or in the Herd Improvement Registry. The records of these cows have been tabulated and converted to a mature yearly equivalent basis using the A.J.C.C. conversion factors. Table 2 shows the records of all the cows that have been classified, grouped according to classification ratings and table 3 shows the same information on the cows classified during the calendar year 1937.

TABLE 2  
*Production records of all animals officially classified*

Classification groups	Number of cows classified	Number of cows with R. of M. or or H.I.R. records	Per cent of cows with records	Average mature production of each group
Excellent	302	228	75.50	650.05
Very Good	983	649	66.09	624.03
Good Plus	1733	991	57.18	602.61
Good	1212	597	49.26	586.97
Fair	142	63	44.37	589.49
Poor	11	1	9.09	556.54
Totals	4383	2529	57.68	608.44

The information in the foregoing tables has been previously published based on a much smaller number of records and attention has been called to the relationship between the ratings of the animals and the production records. It will be observed in examining Table 2, that with the exception of the "Good" and "Fair" groups there is a gradual rise in the production

**TABLE 3**  
*Production records of all animals classified during 1937*

Classification groups	Number of cows classified	Number of cows with R. of M. or or H.I.R. records	Per cent of cows with records	Average mature production of each group
Excellent	103	57	55.34	634.30
Very Good	379	169	44.59	619.47
Good Plus	649	237	36.52	588.67
Good	455	100	21.98	555.61
Fair	54	17	31.48	578.27
Poor	7	1	14.29	556.54
Totals	1647	581	32.49	596.06

as the classification ratings increase. This increase is not large, but it is very significant that a much greater percentage of the higher rating animals have production records than the lower rating animals. Undoubtedly if an equal percentage of each classification group had been tested the differences in production would be much more pronounced. This very fact makes a detailed analysis of the production records difficult. It seems safe to assume that in general, the animals tested represent the better producers within that group. There were forty-four per cent of the animals rated as "Fair" with production records. Table 4 was compiled on the basis of using records on just forty-four per cent of the cows in each classification group and selecting the cows with the highest records. For example, there were 302 cows classified as "Excellent." Forty-four per cent of this number is 133 and of the 228 "Excellent" cows with records, the high 133 were used and their records averaged. This table is obviously subject to considerable error but it does illustrate that if an equal percentage of each classification group had been tested, the differences in production would be much more pronounced than shown in Table 2 or 3.

**TABLE 4**  
*Hypothetical data showing average production when the number of records used is reduced to 44% of the number classified in each group*

Classification groups	No. of cows classified	Per cent with records	No. of cows with records used	Average mature production of each group
		<i>Per cent</i>		<i>lbs.</i>
Excellent	302	44	133	723.41
Very Good	983	44	433	695.74
Good Plus	1733	44	763	654.75
Good	1212	44	533	613.48
Fair	142	44	63	589.49

It has been suggested that the range in production within each classification group exceeds the differences between the various groups and that therefore the relationship between classification ratings and production is not at

all significant. To determine the range in production of the animals tested in each classification group, the following frequency Table 5 was prepared.

TABLE 5

*Frequency table showing distribution of records made by cows classified in various classification groups*

Production divisions	Excellent	Very good	Good plus	Good	Fair
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Over 1000 lbs. of fat ..	0.88	1.69	0.91	0.68	1.59
900 to 999 lbs. ....	2.63	1.23	2.83	2.01	3.18
800 to 899 lbs. ....	7.01	7.41	6.26	3.02	3.18
700 to 799 lbs. ....	22.81	18.05	12.31	14.74	7.93
600 to 699 lbs. ....	31.14	25.62	24.82	22.28	25.40
500 to 599 lbs. ....	25.44	26.45	29.07	29.99	31.75
400 to 499 lbs. ....	8.33	16.05	17.86	19.26	17.46
300 to 399 lbs. ....	1.75	2.47	5.15	6.87	9.53
Below 300 lbs. ....	0	0.93	0.81	1.17	0

Examination of this table does illustrate that there is a wide range in producing ability within each classification group. Summarizing the results show that sixty-four per cent of the "Excellent" animals with records are above 600 pounds of fat, fifty-four per cent of the "Very Good" cows exceed 600 pounds, forty-seven per cent of the "Good Plus" cows exceed 600 pounds in production and forty-three per cent of the cows rated "Good" are over 600 pounds. Of the cows rated "Fair," about forty-one per cent exceed 600 pounds in production. However, the variation in production within each classification group is so marked that a classification rating alone is not of a great deal of value in estimating the producing ability of an individual cow. It seems obvious that a single animal cannot be selected from any one classification group with reasonable assurance that the animal selected will be better or worse in production than an individual animal selected from any other group.

In comparing the "Excellent" animals with records with those classified as "Very Good" having records and doing this at random for the entire group, it was found that in fifty-eight per cent of the times, the "Excellent" cows had higher records than the "Very Good" cows. On the same basis, the chances were fifty-six out of one hundred that a "Very Good" cow would have a higher record than a "Good Plus" cow. The chances were fifty-three out of one hundred that a "Good Plus" cow would excel a "Good" cow and the possibility of a "Good" cow exceeding a "Fair" cow in production was fifty-two times out of one hundred chances. The odds on an "Excellent" cow having a higher record than a cow classified as "Good," were found to be about sixty-three to thirty-seven or sixty-three times out of one hundred comparisons, an "Excellent" cow will exceed a "Good" cow in production.

The possibility of any ten animals picked at random in any classification group, excelling any ten others selected at random from a different classifica-

tion group was of interest and such selections were made five times. In making these selections, ten animals were picked at random from each classification group and compared with ten animals picked at random from the other classification groups. The only provision was that no two animals in any one group be by the same sire. The results are shown in Table 6.

TABLE 6

*Chart showing average yield of 10 records selected at random from each classification division*

Classification	Group 1 (Average of 10 records)	Group 2 (Average of 10 records)	Group 3 (Average of 10 records)	Group 4 (Average of 10 records)	Group 5 (Average of 10 records)
Excellent .....	645	663	612	595	718
Very Good .....	583	617	686	587	688
Good Plus .....	591	528	509	567	579
Good .....	557	539	519	585	532
Fair .....	555	543	603	582	630

It also seemed pertinent to ascertain if the production records of the cows classified were affected by the age at classification. In other words, would animals rated "Excellent" as mature cows be apt to have any higher records than animals rated "Excellent" as immature cows or as old cows past their prime of life. All of the animals classified with production records were divided into three groups. The first group consisted of those cows classified at under five years of age. The second group was composed of animals classified at from five to nine years of age, inclusive, and the third group was composed of cows classified at ten years of age or older. The average production for each age group and for each classification was determined and the results are given in Table 7.

The results shown in this table indicate that age at classification is not a factor affecting the production records of the animals classified. This result was expected for in making the official classifications, the judges are not influenced to any extent by the fact that an old cow may have previously completed an exceptionally high record. The sole guide for classification work is the scale of points of the American Jersey Cattle Club.

It was next determined that a total of fifty bulls have been classified, each having ten or more officially classified daughters. The average score of each bull's daughters was then computed by assigning an arbitrary score of 95.00 for each daughter rated "Excellent," 87.50 for each daughter classified "Very Good," 82.50 for each daughter classified "Good Plus," 77.50 for each daughter rated "Good," 72.50 for each daughter classified "Fair" and a score of 60.00 for each daughter rated as "Poor," in conformation. There were ten bulls rated "Excellent" each with ten or more classified daughters and the average score of all the classified daughters was 85.44 per cent.

TABLE 7  
*Production records of cows classified at various ages*

	Excellent No.    Av. yield	Very good No.    Av. yield	Good plus No.    Av. yield	Good No.    Av. yield	Fair No.    Av. yield
Cows classified at under 5 years of age .....	73    658.03	261    634.91	509    603.71	352    585.96	35    570.60
Cows classified at from 5 to 9 years of age .....	103    653.87	287    616.10	396    599.14	210    590.56	22    625.18
Cows classified at 10 years of age or over .....	52    632.02	101    615.41	86    614.84	35    580.29	6    570.17
Totals .....	228    650.22	649    623.57	991    602.93	597    587.26	63    589.62

There were eighteen bulls rated "Very Good," each with ten or more classified daughters, the daughters having an average score of 83.78 per cent. There were twenty bulls rated "Good Plus" in this category and the average score of their daughters was 81.57 per cent. Finally, there were two bulls classified as "Good," each with ten or more classified daughters and the average score of the daughters was 80.72 per cent.

These results may or may not indicate anything. The numbers are entirely too few to be reliable but from the limited number, it does appear as though there might be some relationship between the type or conformation of a bull and the type and conformation of his daughters. However, it must be remembered that in all probability, the better type bulls were bred to better type cows, while the bulls rated in the lower classification groups were probably bred to cows not so good in conformation.

There were also a number of classified bulls that had qualified as "Tested Sires" with ten or more tested daughters. Likewise, a number of the cows classified have qualified as "Tested Dams," each with three or more tested progeny. These bulls and cows were divided into groups, depending upon their classification ratings and the average yields of their tested progeny obtained. The results of this phase of the analysis are given in Table 8.

TABLE 8

*Classified bulls and cows qualifying as tested sires and as tested dams*

Classification groups	Bulls		Cows	
	Number	Av. yield of tested progeny	Number	Av. yield of tested progeny
Excellent . . . . .	13	635	9	619
Very Good . . . . .	17	607	21	607
Good Plus . . . . .	17	619	38	632
Good . . . . .	6	706	14	598
Fair . . . . .	—	—	1	637

In this table also, the numbers are unfortunately too limited to be dependable but it is significant to note that both with the cows and bulls, there does not seem to be any distinct relationship between the classification ratings of the animals and the production of their progeny. There were several bulls and cows in the "Excellent" group with high producing and high classifying progeny. Such animals are the ideals which breeders are seeking and it is on such cows and bulls that breeders must depend for future breed improvement. Obviously, as classification work increases from year to year, further data will eventually be available but until that time, few definite conclusions can be drawn concerning the relationship between the type of an animal and either the type or the production of the progeny.

## SUMMARY

In reviewing and summarizing the entire problem, it is very unfortunate that the numbers are so limited and also that an equal percentage of the cows classified in each group were not tested. If every animal classified had an official production record, the conclusions would then certainly be important in adding to our knowledge of breeding better dairy cattle. The results however do indicate that there is definitely some relationship between the conformation of a cow and her producing ability and that both good conformation and high production can be combined in the same animal. They are certainly not inimical to each other. On the other hand, it seems a fallacy to suppose that by breeding solely for production, we may secure ideal breed type or that by breeding solely with type in mind, we can secure the ideals in production. Breeders must continue to select and breed with both ideals in mind and only by following such a practice can our dairy breeds be definitely improved and made more uniform in conformation and producing ability.



# SWEETENED CONDENSED WHEY: ITS MANUFACTURE AND PROPERTIES

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Whey, as a by-product in cheese manufacture, is produced in relatively small quantities in widely scattered factories. A soluble, edible, dry whey can be made in large cheese producing areas where sufficient material is available to operate a spray drying unit. But since whey contains approximately 93 per cent water and is an excellent medium for bacterial growth, it cannot be profitably shipped long distances for processing. The small cheese factory which is not located near a suitable drying plant must either return its whey to the farmer for hog feed or discard it.

Sweetened condensed whey was developed in the hope that it might help to fill the need for a cheap and simple method for preserving whey for human food. The new product is essentially sweetened condensed milk with the casein and milk fat removed.

Sweetened condensed whey has many possible uses in food preparations, but since work on this phase of the subject is still unfinished details will appear in a later paper. The mixture may be added to any food where both whey solids and sugar are desired. It has been used experimentally in fruit jams and whips, certain bakery products, and as an ingredient for several types of candy. Sweetened condensed whey has excellent whipping properties but without flavoring it is not pleasing to the taste. However, with the addition of suitable flavoring material, the product should be useful at soda fountains as a topping for hot chocolate, sundaes, cakes, and similar foods.

## EXPERIMENTAL

Work was conducted to determine the feasibility of preserving whey solids with sugar; the optimum quantity of sugar to use; the most satisfactory total solids value for the condensed product; the effect of storage upon viscosity; the value of including butterfat or coagulated whey protein in the concentrated mixture; and finally to investigate any special properties which might enhance the use of the material in food products.

Swiss and cheddar cheese whey and rennet casein whey were available for the work. Cane sugar was used as the preserving agent except where glucose or invert sugar are specifically mentioned. Unless otherwise stated, the whey which was used in all experiments was centrifugally separated to remove the butterfat left in the cheese making process.

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The process of manufacture employed for making sweetened condensed whey was somewhat similar to that used in producing sweetened condensed milk. The whey was pasteurized at 62° C. for 30 minutes, the required quantity of sugar added, and the mixture evaporated in an 18-inch tinned copper pan under 26 to 28 inches vacuum. The condensed product was cooled to 35° C. and stirred for 3 or more hours at room temperature. Very small lactose crystals were essential in the preparation of a smooth product.

Samples of sweetened condensed whey were stored in airtight containers for observation of keeping quality and change in viscosity. Unless otherwise noted, storage was at room temperature, which varied between 25° C. and 30° C.

Viscosity determinations were made with a McMichael viscosimeter at 25° C. Readings were recorded as soon as they remained practically constant. This point was generally reached after the viscosimeter cup had revolved for 5 minutes. Sweetened condensed whey, like many dairy products, developed a structure during storage which partly broke down with stirring. The products were stirred uniformly to adjust the temperature and to secure a representative sample. Some breakdown of structure was inevitable. The figures represent relative rather than true viscosity values but were suitable for comparative purposes. No attempt was made to differentiate plastic from viscous flow.

Apparent viscosity values were calculated in poises from the degrees McMichael on the instrument dial. Wires ranging from No. 18 to No. 30 were used with either the small or large plunger, depending upon the viscosity of the material under investigation. The wires and plungers were calibrated in accordance with instructions furnished with the instrument.

Overrun was determined by whipping sweetened condensed whey with an electrically operated household mixer, using high speed running at 1,000 r.p.m. without a load. Percentage overrun was calculated as 100 times the difference in weight of the same volume before and after whipping divided by the weight after whipping.

## RESULTS

Attention was first focused upon the quantity of sugar sweetened condensed whey should contain. Preliminary experiments showed that in the case of concentrated whey with its high salt content, a 58 to 60 per cent sugar/sugar + water ratio was sufficient to retard bacterial growth. The ratio was calculated as follows:  $\text{Per cent. sucrose in sucrose + water} = (\% \text{ sucrose} \times 100) \div (\% \text{ sucrose} + \% \text{ water})$ . The quantity of sugar necessary to give a product of good keeping quality was low enough to allow considerable latitude in the total solids range of the condensed material. The practical limits for sweetened condensed whey were found to be between 70 per cent and 80 per cent total solids with the optimum at 76 per cent.

The proportion of whey solids to sugar has been expressed as the per cent whey solids ÷ per cent sugar or the W/S ratio. From the W/S ratio and the total solids (W + S) of a sample the percentages of whey solids, sugar, and sugar in water may be calculated. If W + S = 79.2 and W/S = 1.34 (Table 1), then  $W = 79.2 - S$  and  $\frac{79.2 - S}{S} = 1.34$  and S = 33.8%.

Data given in Table 1 show the effect of increasing the proportion of whey solids to sugar upon the viscosity of sweetened condensed whey. Each

TABLE 1

*Effect of variations in the whey solids/sugar ratio upon the viscosity of sweetened condensed whey held at room temperature*

W/S ratio	Total solids	Viscosity after 2 months
	<i>Per cent</i>	<i>Poises</i>
0.80	80.3	59.0
1.00	78.8	102.1
1.01	79.6	698.7
1.18	79.9	834.3
1.34	79.2	1,677.0

set of figures represents a different batch. The total solids of each run approximates 80 per cent, which is the upper practical limit to which the product may be concentrated. The viscosity figures therefore represent maximum viscosities for each W/S ratio after two months storage. For economical preservation a large W/S ratio was advantageous, but after the viscosity of the mixture exceeded about 800 poises the product became too heavy for general use. The values for the sugar in water concentration of the samples shown in Table 1 ranged from 72.6 per cent for the low whey batch to 61.9 per cent for the product with a W/S ratio of 1.34. If the mixtures had been condensed to 76 per cent instead of 80 per cent total solids, it would have been necessary to increase the quantity of sugar to obtain the same sugar/sugar + water concentration.

From the foregoing considerations it became evident that the simplest and most practical concentration of ingredients for sweetened condensed whey was approximately: Sucrose 38 per cent, whey solids 38 per cent, and water 24 per cent. Such a mixture had a sugar/sugar + water ratio of about 61 per cent. The best manufacturing procedure for this product was as follows:

The total solids content of the fresh whey was determined according to Sanders' formula (1),  $0.24 \times L + 1.2 \times \text{per cent fat}$ , where L = the lactometer reading at 25° C. (Quevenne or Sp. Gr. scale). If the lactometer reading of separated whey was 29.17 then  $29.17 \times .24 + (1.2 \times 0) = 7.0\%$  whey solids. Then to each 100 pounds of fresh separated and pasteurized whey of 7.0 per cent solids was added 7 pounds of cane sugar. The mixture was con-

densed under vacuum to 76 per cent solids. At this point the specific gravity was 1.360 at 50° C. or 1.365 at 40° C. The mixture was cooled to 35° C and stirred at room temperature for at least three hours. It was then held in sealed containers to prevent mold growth.

After the establishment of the optimum W/S ratio at 1.0 this factor was held constant in most of the subsequent work.

Data plotted in Figure 1 show the rate of increase in the viscosity of fresh sweetened condensed whey as the percentage of total solids was in

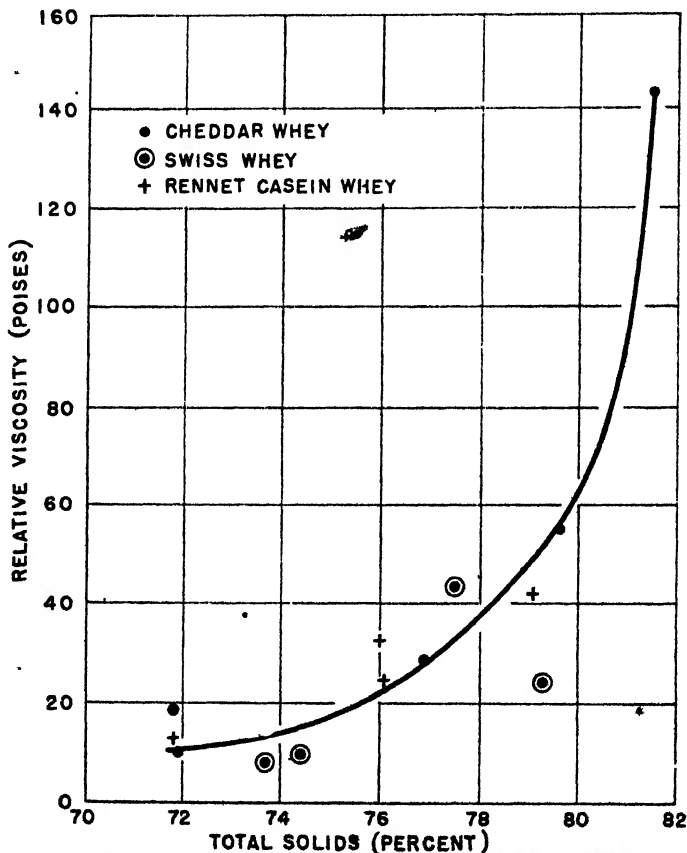


FIG. 1. The relationship between the total solids content of sweetened condensed whey and its viscosity.

creased by evaporation of water. Each point represents a result obtained on a separate batch made from different whey. The distance of some of the points from the curve emphasizes the difficulty which was experienced in obtaining accurate viscosity measurements, but it was felt that the data reflected the true rate of increase of relative viscosity with total solids.

The temperature of pasteurization of whey influenced the viscosity of the condensed product. Data for three temperatures are presented in Table 2.

TABLE 2

*Age thickening of sweetened condensed whey pasteurized for 30 minutes at different temperatures*

Pasteurization temperature	Total solids*	Viscosity after aging at room temperature			
		2 days	13 days	47 days	116 days
°C.	Per cent	Poises	Poises	Poises	Poises
55	75.0	17.5	18.9	25.4	44.9
62	74.9	18.4	23.1	26.0	32.1
68	75.8	29.3	27.4	42.1	73.3

\* Made from unseparated cheddar whey, the total solids thus including 1.3 per cent butterfat in each case. The whey solids-sugar ratio = 1.0.

The difference in viscosity between samples pasteurized at 55° C. and 62° C. was of little significance. The greater age thickening of the whey pasteurized at 68° C. was probably caused by denaturation of some of the whey protein during pasteurization. When it was important to preserve the original solubility of the protein, pasteurization temperatures of 68° C. or higher were not desirable.

Sweetened condensed whey darkened in color as the temperature or time of storage was increased, but the brown color did not become excessively dark during two or three months storage at room temperature.

The lactose concentration in sweetened condensed whey was approximately 2½ times the amount present in sweetened condensed milk, consequently it was to be expected that a settling of lactose crystals would occur in the whey during long storage periods. The amount of separation, which varied with crystal size and the viscosity of the mixture, was not an important influence upon the quality of the product.

The total solids content of sweetened condensed whey was an important factor in its age thickening. In Table 3 data are given to show the effect of

TABLE 3

*Effect of concentration on age thickening of sweetened condensed whey held at room temperature\**

Total solids	Viscosity after aging					Average daily increase
	1 day	7 days	40 days	82 days	142 days	
Per cent	Poises	Poises	Poises	Poises	Poises	Poises
71.9	9.9	18.8	35.4	33.5	33.5	0.17
76.9	28.4	38.8	80.3	121.2	363.3	2.36
81.5	144.1	162.6	894.4	978.2	1257.7	7.84

\* Cheddar whey in which the whey solids/sugar ratio = 1.0.

variations in total solids from 71 per cent to 81 per cent upon relative viscosity during aging. The increase in viscosity of the sample containing 76.9 per cent solids was typical of the thickening of most of the batches con-

densed to approximately this concentration. A few samples did not show a uniform viscosity increase during aging while some others (Table 2) did not increase as rapidly as the wheys of Table 3. Although a considerable variation in viscosity development during storage can be anticipated, especially when storage temperatures fluctuate, the normal viscosity increase which develops at cool room temperatures should not detract from the usefulness of the product.

Just as the viscosity of sweetened condensed milk was influenced by storage temperature, so also was this true for that of sweetened condensed whey. However, it was not intended that sweetened condensed whey should require cold storage temperatures, especially since the viscosity which it developed at ordinary temperatures was not detrimental. A thorough investigation of the influence of storage temperature was not made. However, the following figures were considered to represent the increase which would probably be encountered in the viscosity of sweetened condensed whey stored at different temperatures. The figures were obtained from measurements of a whey of 76.9 per cent total solids and a W/S ratio of 1.03. The whey solids included 2.4 per cent of coagulated whey protein added to increase the protein content of the mixture. The holding period was 97 days.

Storage temperature in °C.	Viscosity in poises
Check, not aged	34.0
2	44.9
10	52.0
20	61.4
Room	70.9
37	85.1

The addition of the coagulated whey protein raised the viscosity only slightly above that of most normal batches. While it was not deemed necessary to keep sweetened condensed whey in cold storage to retard an increase in viscosity, it was found desirable to store the product in a cool place to minimize the tendency toward thickening.

Sweet wheys containing butterfat or extra protein were investigated because of the possibility of their use in special food products where quantities of these ingredients were required. However, these additions increased the cost.

Experimental lots of sweetened condensed whey containing butterfat were made. Whey cream was added to give a final fat content of 6 to 14 per cent. This product when condensed to 75 to 80 per cent solids was quite viscous. It remained of good body and flavor during storage at room temperature for three or four weeks, but beyond this time a deterioration in butterfat flavor was noticeable. Some interesting data on the effect of replacing water by butterfat in concentrated sweetened whey are given in

Table 4. The viscosity of condensed whey was greatly increased by the addition of butterfat.

TABLE 4

*Effect of replacing water by butterfat on the viscosity of sweetened condensed whey\* held at room temperature*

Total solids	Butterfat	Solids not fat	Viscosity after 2 months
<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Poises</i>
73.3 . . .	0.0	73.3	6.6
87.0 . . .	13.9	73.1	1816.7

\* Whey solids/sugar ratio = 0.56.

The presence of small quantities of butterfat in sweetened condensed whey destroyed the whipping properties of the product. When the whey was not separated before condensing, as in the samples mentioned in Table 2, the small percentage of fat kept well during storage but it rendered the product unfit for use where whipping in air was desirable.

Several batches of concentrated whey were made to which extra whey protein was added. Heat coagulated protein was obtained by heating fresh whey and removing the coagulated protein which was at once redispersed by homogenization in a new lot of hot fresh whey. Sugar was added and the mixture condensed in the usual manner. The physical properties of this product were found to be little different from those of the normal condensed material. Data on the viscosity of a batch of this material held at different temperatures have been given above.

Both corn and invert sugar were substituted for part of the cane sugar in sweetened condensed whey according to the requirements of the food product in which the whey was to be used. However, the sucrose could not be entirely replaced by corn sugar because of the limited solubility of dextrose at room temperature. Invert sugar or honey, on the other hand, completely replaced cane sugar to give a sweeter whey, but for most uses only a partial substitution was necessary.

The general principles of the work of Ramsey, Tracy, and Ruehe (2) on the use of corn sugar in sweetened condensed milk were found to apply to sweetened condensed whey. The product became excessively brown during processing and storage when most of the sucrose was replaced by either corn or invert sugar. When dextrose or invert sugar was substituted for sucrose up to 50 per cent a satisfactory sweetened condensed whey was obtained.

One of the most promising features of sweetened condensed whey was the ease with which it could be whipped. The foam-producing whey protein permitted the incorporation of air during whipping while the high viscosity of the product aided greatly in stabilizing the whip.

The increase in volume during the whipping of sweetened condensed

whely of different solids content is shown in Table 5. A whey sample containing 79 per cent total solids was diluted with water to various concentrations and whipped until maximum overrun was attained as determined by the point where further whipping did not incorporate additional air. The

TABLE 5  
*Effect of total solids upon the whipping properties of sweetened condensed whey.*  
*Whey/solids sugar ratio = 1.0*

Whipping time at 30° C.	Overrun produced by wheys of different total solids content					
	Total solids content—per cent					
	79	75	70	65	60	50
<i>Min.</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
1	84	72	67	101	93	27
2	138	143	127	121	159	
3	179	194	188	191	214	35
4	182*	213*	229	240	293	
5	179	213	254	281	340	39
6			259*	300*	358	
7			259	300	368*	43
8					368	
9						46
11						54*

\* Stability of whip at maximum overrun. Temperature 28°–30° C.

<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>M'</i>
3600	910	205	130	70	0

stability of the whips also is given in Table 5. This was determined as the time when the first drainage appeared in the bottom of a 150 cc. glass filled with whipped material.

It was not possible to obtain a stable whip when the total solids were 50 per cent. This mixture was too low in viscosity to have even slight stability. The sample containing 79 per cent solids was the most viscous and showed the greatest stability. The highest overrun was produced by the 60 per cent solids sample which was thin enough to allow an easy incorporation of air and at the same time viscous enough to hold it, at least for a short period.

For practical considerations it may be said that aging does not significantly influence the whipping properties of sweetened condensed whey.

The data of Table 6 show that approximately the same maximum overrun was finally obtained during whipping regardless of the aging period with its accompanying increase in viscosity. These data seem to indicate again that the increase in viscosity with age was largely an increase in apparent viscosity, hence the difficulty experienced in securing accurate viscosity measurements. As the aging period progressed and the viscosity increased (Table 6), the overrun for the first two minutes of whipping showed a de-

TABLE 6

*Effect of aging upon the whipping properties of sweetened condensed whey\**

Whipping time	Overrun after different aging periods			
	Fresh	28 days	51 days	73 days
<i>Min.</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
1 .....	113	101	105	98
2 .....	202	191	174	172
3 .....	235	229	229	238
4 .....	248	249	232	259
5 .....	238	225	230	242
Viscosity of aged samples before whipping				
<i>Poises</i>	<i>Poises</i>	<i>Poises</i>	<i>Poises</i>	<i>Poises</i>
9.4	17.9	35.4	49.6	

\* Aged at room temperature. Measurements at 25° C. Whey solids/sugar ratio = 1.0. Total solids = 74.4 per cent.

crease. Apparently about two minutes of vigorous whipping was required to beat out the temporary viscosity which had developed.

Investigation was made of the effect of heating to various temperatures upon the whipping properties of sweetened condensed whey. Five hundred gram samples of 76 per cent solids sweetened condensed whey were heated to 70° C., 80° C., 90° C., and 95° C. for 5 minutes. The water loss was replaced, the samples cooled to room temperature and held at 23° C. for 24 hours with occasional stirring to recrystallize the lactose. The wheys were then whipped to their maximum overruns in 4 minutes for the unheated check and in 3 minutes for the heated samples. The overruns obtained were: Check, 144 with 180, 153, 147, 171 per cent in the order of increasing temperatures of heating. The whipped check sample showed its first drainage in 3 days, the 95° C. sample not until 5 days, with the others falling between these times. The data indicate that heat coagulation of the soluble whey protein is not detrimental to the whipping properties of the sweetened condensed product. Evidently the whipping properties of this material are dependent upon its high viscosity and, at least partially, upon a non-heat coagulable foam-producing material identical perhaps with that described by Ansbacher, Flanigan and Supplee (3).

## SUMMARY

1. Whey solids were simply and inexpensively preserved in a form directly utilizable in sweet foods by condensing a mixture of fresh whey and sugar.

2. The most satisfactory procedure was to add to separated, pasteurized whey a quantity of sugar equal to the weight of the whey solids. The mixture was condensed under vacuum to 76 per cent total solids, cooled to 35°

C. and stirred for at least 3 hours to produce small lactose crystals. It was sealed in airtight containers.

3. The effects of manufacturing processes, of concentration of ingredients, and of storage conditions upon the relative viscosity of sweetened condensed whey were determined.

4. Sweetened condensed whey kept well during storage at room temperature for at least 3 months. The changes during such a storage period were a slight darkening in color and a small increase in viscosity. Holding at cool temperatures minimized the changes which occurred during storage.

5. Sweetened condensed whey was easily whipped to an overrun of approximately 200 per cent in 4 minutes. The whip was stable for 15 hours. The relationship between total solids content, overrun, and stability of whip was investigated.

#### ACKNOWLEDGMENT

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# SOME EFFECTS OF A VITAMIN D DEFICIENCY ON MATURE DAIRY COWS

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The important role played by vitamin D in preventing disease and promoting the efficient utilization of calcium and phosphorus by calves and young cattle has been established by the numerous experiments on this problem which have been reported in the last few years. Whether or not a deficiency of vitamin D in the nutrition of mature dairy cows will result in the development of any or all of the deficiency symptoms exhibited by calves under similar conditions has remained as an unanswered question. The possibility of vitamin D improving the utilization of calcium and phosphorus by mature cows so that positive balances of these minerals might be maintained during periods of liberal milk production has been studied by workers at the Wisconsin Station (6, 7, 8, 9, 10, 11, 12, 13). In this series of experiments, green roughages, roughages dried with varying amounts of sunshine exposure, sunlight, ultra-violet light, cod liver oil, and irradiated yeast were studied for their influence on calcium and phosphorus metabolism. They were used either as a part of the ration or supplemental to normal rations carrying different amounts of calcium and phosphorus. Under these conditions the authors were unable to demonstrate any consistent significant influence of vitamin D in improving the utilization of these minerals. Meigs and coworkers (14) also concluded from their work that the addition of cod liver oil to the ration had no favorable influence on calcium assimilation.

The present investigation was designed to study the necessity of vitamin D in the adequate nutrition of mature dairy cows by first depriving them of this factor, watching for the development of any symptoms which might result from a vitamin D deficiency, and then attempting by the administration of vitamin D to relieve or improve such conditions as might develop.

## EXPERIMENTAL METHODS

The essential features of this experiment included the keeping of mature dairy cows under conditions as free from food and environmental sources of vitamin D as possible and making such observations as might reveal the development of any abnormalities commonly associated with a deficiency of this factor.

Five mature, grade Holstein cows have been used so far in this experiment. Three heavy milking cows of this group have supplied most of the data reported in this paper. The cows were kept inside except for occasional exercise periods in a dry lot after dark. Weights were obtained on three

consecutive days once per month. The rations were designed to be adequate except for vitamin D. To accomplish this it was necessary to use molasses beet pulp as the source of roughage as most of the common roughages carry more or less vitamin D. Samples of the beet pulp used were tested biologically in our laboratory and were shown to be free of detectable amounts of vitamin D. A grain mixture of ground yellow corn, ground oats, and corn gluten meal was used to balance the ration. The grain mixture also included common salt and sufficient bone meal to supply what would normally be an adequate amount of calcium and phosphorus. The mineral allowance provided at least 20–25 grams of calcium and 10 grams of phosphorus for maintenance of the cow and in addition 1 gram of calcium and 0.75 gram of phosphorus for each pound of milk produced daily. Additional vitamin A was supplied by a special concentrate which was shown by biological assay to contain no significant amount of vitamin D. The mangers were partitioned off so that each cow could be fed individually and refused feed accounted for on the feed record. Shavings were used for bedding.

Three-day composite blood plasma samples were obtained regularly at monthly intervals for the determination of total calcium and inorganic phosphorus. Additional samples were taken at irregular intervals whenever the condition of the animal indicated that pertinent data might be secured. Calcium was determined by adaptations from the method of Clark and Collip (2) and phosphorus by the Fiske-Subbarow method (4).

Calcium and phosphorus balances were determined in ten-day trials at about monthly intervals and more frequently when made necessary by the condition of the animal. The balance trial rations were fed for several days previous to the start of collection periods. They were weighed out in daily portions and sampled for analysis in advance of the trial. The cows were placed in adjustable metabolism stalls on elevated platforms so that a large pan with removable shield could be placed at the rear to collect the excreta which were aliquotted at regular intervals and composited at the end of the trial. The feed stuffs and excreta were analyzed by methods essentially the same as those described by Morris, Nelson, and Palmer (15).

Butter fat samples for vitamin D assay were obtained by saving all the milk from the cows concerned for a sufficient period of time to give a two-quart jar of pure butter oil after it had been separated, churned, and either filtered or centrifuged to remove the curd. It was stored at about 0° F. until assayed for vitamin D using the standard line-test technique.

Calcium and phosphorus analyses were run on milk samples taken from the colostrum, from a one-day composite at the end of the third day, and from two-day aliquots taken at the fifteenth day, thirtieth day, and at subsequent thirty-day intervals throughout the lactation.

The physical condition of the cows was observed at regular intervals and notations made of any conditions which would be of value in interpreting the

results of the experiment. The customary breeding, health, and milk records were also obtained.

### RESULTS

In a study of this type the conditions and responses pertaining to each individual animal necessarily show some variations. To make the presentation as intelligible as possible the full set of observations and data for each cow will be given separately in this section. The implications of the data as a whole, and generalizations deduced therefrom will be presented later in the discussion section.

*Cow 3E.*—The animal designated as 3E was placed on the vitamin D deficient ration on Dec. 15, 1935. She completed a lactation in March and freshened normally on April 12, 1936. As shown in Chart 1, the total calcium of the blood plasma had been in the normal range of 10–12 milligrams per 100 cc. and the inorganic phosphorus in its normal range of about 5 milligrams per 100 cc. previous to the approach of parturition. No abnormalities were in evidence until two days after giving birth to a robust, 108 pound male calf when the cow seemed to be paralyzed in her back and limbs. She was unable to get up further than onto her knees but could then drag herself around the boxstall to a limited extent. The blood calcium at this time had declined to 5.3 mgm. per 100 cc. of plasma and the inorganic phosphorus to 3.1 mgm. Five hundred cc. of cod liver oil were administered over a two-day period in an attempt to improve her condition. The cow was on her feet the following day and in a few days was eating her feed readily. The rapid increase in the blood calcium and phosphorus is indicated on Chart 1. Milk production increased until she was giving about 62 pounds daily. A recession in the level of blood calcium and phosphorus soon set in again, and about four months after freshening evidences of stiffness began to appear. There was some swelling of the joints and the knees began to spring forward. She walked with the stiffness exhibited by calves suffering from a vitamin D deficiency, and scarcely flexed the joints of her legs. Her backbone became so stiff that she could not bend it to lick herself, neither did she flex it in walking or turning around. She had great difficulty in lying down and getting up and when standing she frequently lifted each foot with a trembling motion evidently to ease the pain by removing the weight from it.

A ten-day balance trial was run, and a sample of butterfat was saved for vitamin D assay just before these conditions became so severe that remedial measures had to be taken. As shown in Table 1, the calcium balance was negative by 24.06 grams and the phosphorus only slightly positive to the extent of 6.97 grams for the ten-day trial. There was no detectable amount of vitamin D in the butterfat sample as was indicated by the fact that five rats from different litters completed the assay requirements satisfactorily,

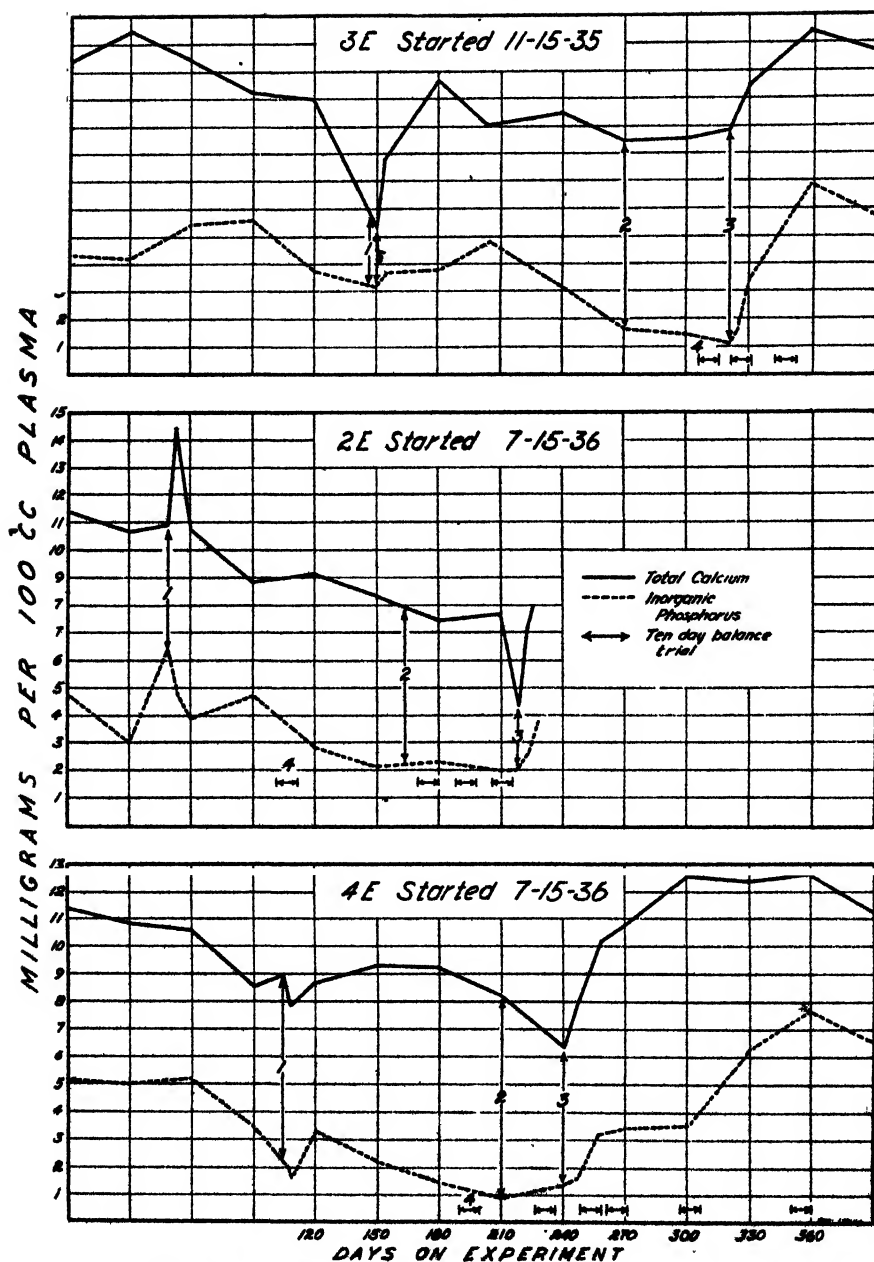


CHART 1. The total calcium and inorganic phosphorus in the blood plasma of mature dairy cows on a vitamin D deficient ration.

1—Time of parturition.

2—First appearance of stiffness.

3—Vioosterol administration started.

4—Spacing of ten-day mineral balance trials.

Results given in Table 1.

5—500 cc. cod liver oil given 3E in a two-day period.

TABLE 1  
The results of ten-day calcium and phosphorus balance trials run on vitamin D deficient cows before and after the administration of vitamin D

Date trial started	Daily milk yield	Ten-day intake		Ten-day balance					
				Before feeding vitamin D		After feeding vitamin D			
		Calcium	Phosphorus	Calcium	Phosphorus	Calcium	Phosphorus		
<i>lbs.</i>				<i>gm.</i>	<i>gm.</i>	<i>gm.</i>		<i>gm.</i>	<i>gm.</i>
				3E 4-12-36 calving					
9-22-36	22.7	480.29	363.41	(-)	24.06	(+)	6.94	(+)	65.10
10-6-36	18.7	390.70	262.20					(+)	230.20
10-10-36	14.3	387.44	227.74					(+)	204.93
11-24-36	13.8	413.89	261.07					(+)	108.01
				2E 9-4-36 calving					
10-27-36	32.9	568.69	393.13	(-)	138.40	(-)	74.94	(-)	39.72
1-5-37	20.1	392.56	269.67	(-)		(-)	89.27	(-)	21.63
1-20-37	16.0	295.01	169.25	(-)	81.06	(-)	54.94	(-)	24.57
2-9-37	13.0	323.91	182.95	(-)	55.96	(-)		(-)	
				4E 11-2-36 calving					
1-26-37	24.9	430.10	283.60	(-)	107.18	(-)	36.49	(+)	131.78
3-2-37	19.8	507.50	277.20	(-)	38.13	(+)	1.67	(+)	239.51
3-23-37	18.9	561.31	296.78					(+)	213.99
4-6-37	19.5	476.00	296.00					(+)	185.38
5-11-37	20.2	496.63	244.91					(+)	102.99
7-6-37	22.5	576.19	343.57					(+)	94.68
Algebraic Totals				(-)	534.06	(-)	203.96	(+)	1270.89
								(+)	601.83

but none of them showed any healing from the twelve grams of fat given each one during the test period.

Five and one-half months after parturition the blood calcium was down to 8.5 mgm. and the phosphorus to 1.1 mgm. per 100 cc., and her distress became so severe that 5 cc. of viosterol were given daily to supply vitamin D. Another mineral balance trial was started on the same day that viosterol therapy was initiated. A third balance trial was started ten days after the close of the second trial. The large calcium and phosphorus retentions which now prevailed as contrasted to the negative balance of calcium and slight positive balance of phosphorus under vitamin D deficient conditions are shown in Table 1. A fourth trial run two weeks later indicated the continued retention of large amounts of calcium and phosphorus. Three weeks after viosterol feeding was started the blood plasma calcium and inorganic phosphorus were back to the normal range again and the physical condition had improved to such an extent that only a slight stiffness could be discerned as she walked.

The calcium and phosphorus in the milk under the conditions of this lactation as compared with the amounts found in a previous normal lactation are shown graphically in Charts 2 and 3. The smoothness and similarity of

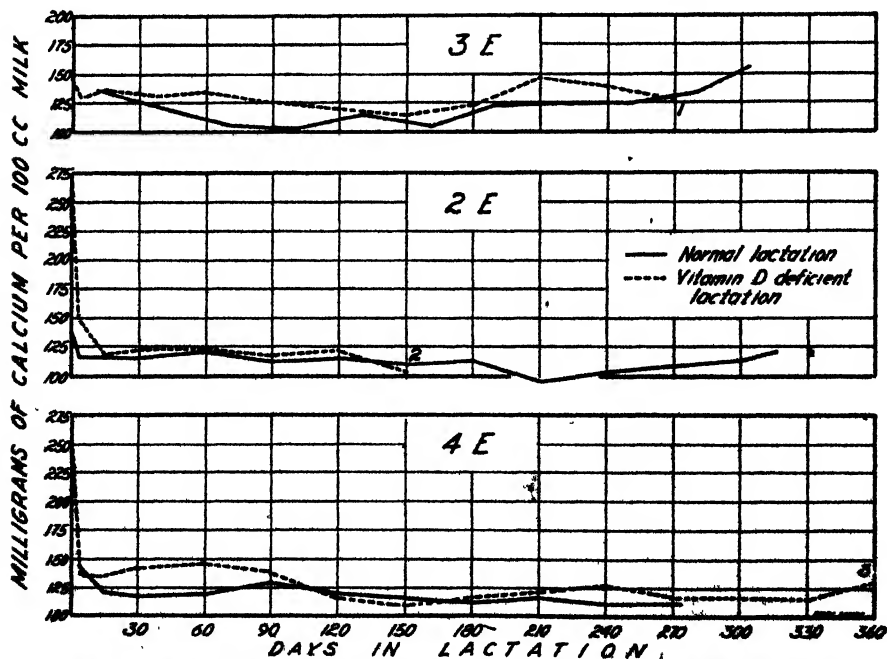


CHART 2. The concentration of calcium in the milk of three cows during a vitamin D deficient lactation as compared with a previous normal lactation.

1—Incomplete lactation—3E sold.

2—Cow 2E died.

3—Lactation still not completed. Delayed pregnancy due to failure of estrum during vitamin D deficiency allowed for extended lactation period.

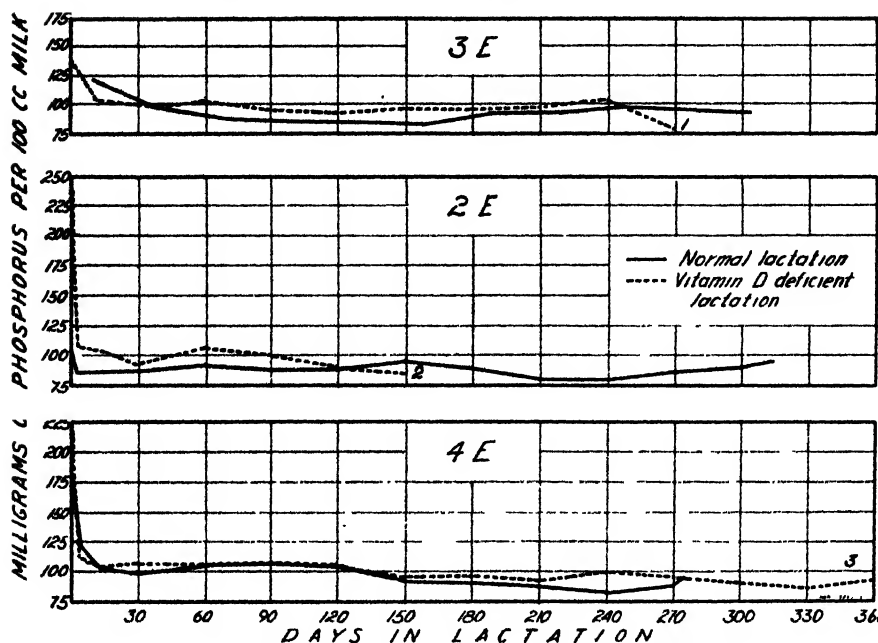


CHART 3. The concentration of phosphorus in the milk of three cows during a vitamin D deficient lactation as compared with a previous normal lactation.

1—Incomplete lactation—3E sold.

2—Cow 2E died.

3—Lactation still not completed. Delayed pregnancy due to failure of estrum during vitamin D deficiency allowed for extended lactation period.

the two curves in each case gives no indication that the level of calcium and phosphorus in the milk was affected significantly by the deficiency of vitamin D. A study of the percentage of calcium and phosphorus in the milk obtained in balance trials run before and after vitamin D therapy also substantiates this view. In the balance trials run under vitamin D deficient conditions the concentration of calcium in the milk was 0.1112 per cent and of phosphorus 0.0964 per cent, while in the two trials run immediately after feeding the vitamin D the calcium percentages were 0.1162 and 0.1091 respectively, and the phosphorus 0.0947 and 0.0876, respectively. Although the composition of the milk was not changed with regard to its calcium and phosphorus content, there was, however, a pronounced decline in the quantity produced, the drop from the high level of production in early lactation being much more rapid than for a comparable normal lactation.

Cow 3E was never observed to show estrum during the vitamin D deficient lactation although her breeding record previous to this time was very commendable. She was reported suspicious to the abortion test and was sold while still in milk nine months after calving.

*Cow 2E.*—This animal was transferred from a normal ration to the vitamin D deficient ration during the first week of July, 1936. After eight

weeks on this ration she gave birth to a strong, 101 pound male calf on Sept. 4. No abnormalities were in evidence although the blood plasma calcium and inorganic phosphorus had been declining previous to parturition and fluctuated conspicuously during the calving interval as is indicated in Chart 1. She was soon eating well and giving up to 42 pounds of milk daily. The blood plasma calcium and inorganic phosphorus continued to decline and on Oct. 27, about seven weeks after freshening the first mineral balance trial was run. There was no evidence of stiffness at this time. The large negative balances of both calcium and phosphorus are shown in Table 1. By the last of December, 15 weeks after freshening, stiffness was developing, the knees were swelling, and the back was elevated and held rigid as she moved about with a stiff, shuffling gait. Three more balance trials were run in rapid succession as shown in Table 1. During this time her physical condition became gradually worse so that by the end of the fourth trial she needed assistance to get to her feet although she was still in fair flesh. Her appetite was not keen and during the last two trials the mineral intake fell slightly below the desired intake to meet the calculated requirements for maintenance and milk production. Negative balances of calcium and phosphorus prevailed in all of these balance trials.

During the time that the vitamin D deficiency symptoms were pronounced a butterfat sample was saved for assay, but it was impossible to detect any vitamin D as indicated by the fact that the seven rats receiving 12 grams of this fat showed no signs of healing except for a mere trace on one animal. A photograph was also taken to show how her knees were buckled forward. This picture is shown in Figure 1. On February 23, about 23 weeks after freshening, and following the completion of the fourth balance trial, an attempt was made to secure a few more feet of moving pictures to supplement those previously taken to show the movements characteristic of this deficiency, but the animal was unable to get up even with the help of three or four men. She showed some tetany after slight exertions. A blood sample was taken in which the calcium was found to be only 4.35 mgm. and the inorganic phosphorus 2.00 mgm. per 100 cc. of plasma. Five cc. of viosterol were given in a drench and this was continued twice daily for the next five days. The rapid improvement in the blood plasma calcium and inorganic phosphorus is indicated in Chart 1. Although she was unable to get up, she continued to look bright and alert, and to eat some for three or four days. Gradually, however, her eyes dulled, her ears began to droop, and she became more and more listless until she died on March 1, six days after she was first unable to get up.

The Veterinary Department assisted with a post mortem examination in which it was found that both femur bones had been broken. Large hemorrhagic areas were present throughout the region of the hip and thigh muscles. Some fiber formation had taken place and the muscle tissues were decomposing, indicating that the bones had been broken for several days. This

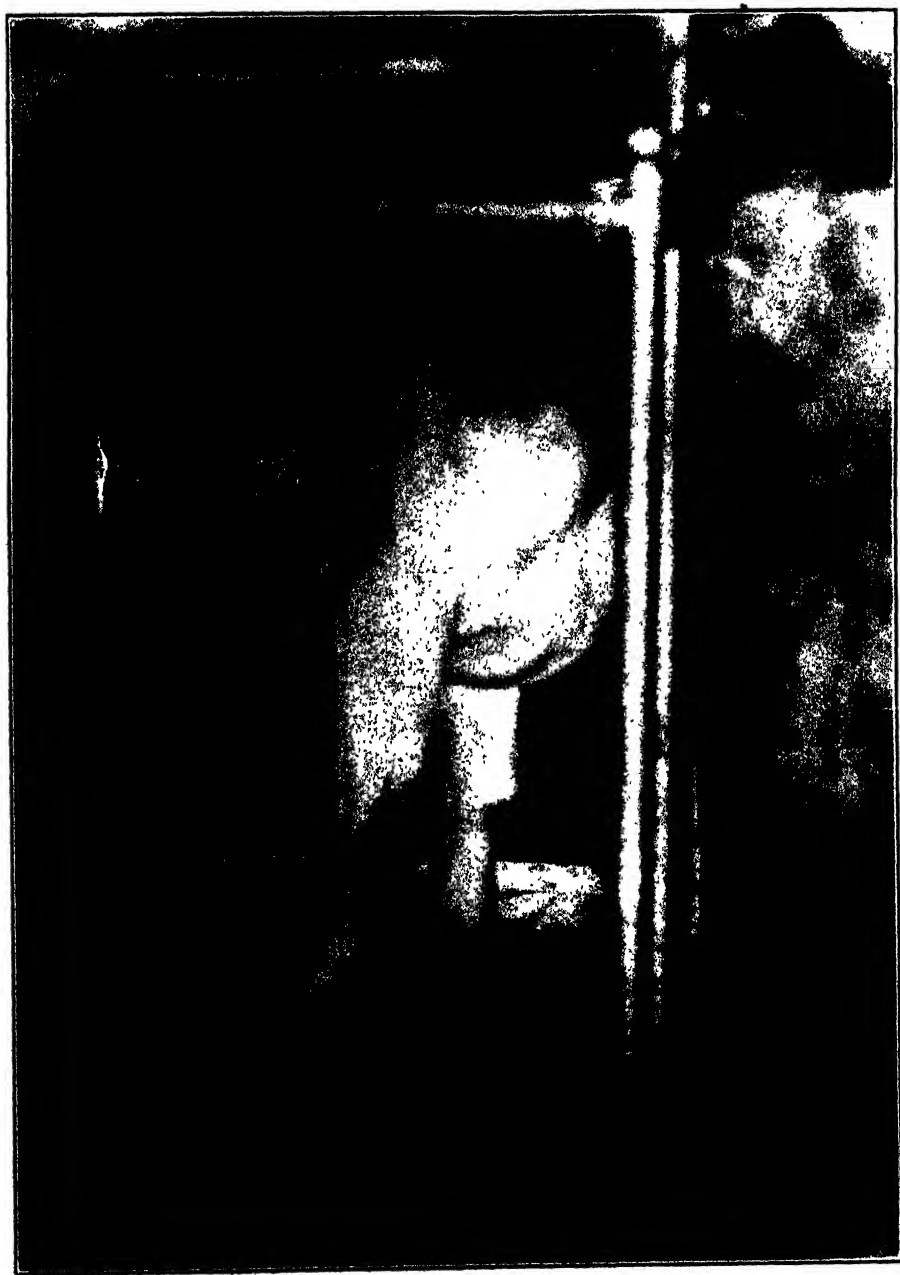


FIG. 1. A vitamin D deficient cow, 2E, showing the knees bent forward.

observation also harmonizes with observations made in the barn notes by the man attending the animal. The broken end of one femur had punctured the abdominal wall. It was quite evident that generalized septicemia had been the immediate cause of her death. The bones must have been uncommonly brittle as likely they were broken sometime when the cow was attempting to get up. She was never seen to struggle violently at any time. One can only conjecture as to whether or not the vitamin D deficiency played any part in making the bones fragile but it would seem quite possible that it may have contributed to this condition. A picture of these bones is shown in Figure 2.



FIG. 2. Broken femur bones from a vitamin D deficient cow, 2E.

The articulating surfaces of the joints seemed to be smooth and normal except for an eroded area about  $\frac{3}{4}$  inch in diameter on the proximal end of the right humerus. The ovaries seemed normal, but one of them contained a corpus

luteum which may account for the fact that this animal never showed estrum during this lactation although per previous breeding record was entirely regular.

The data shown in Charts 2 and 3 indicate that the amounts of calcium and phosphorus in the milk produced under the severe vitamin D deficiency conditions of this lactation were not essentially different from the amounts contained in the milk of a previous normal lactation. As it was not possible to run a balance trial after vitamin D administration, a comparison of the calcium and phosphorus concentration in the milk before and after such a change in the schedule is not possible in this case.

*Cow 4E.*—This animal was also started on the vitamin D deficient ration early in July of 1936. Chart 1 shows that there was a steady downward trend in the blood plasma calcium and inorganic phosphorus which was accentuated the last six weeks before parturition which occurred on November 4, 1936. The 90 pound heifer calf seemed well developed, bright, and alert, but its legs were badly crooked, giving it an outward appearance of a rachitic condition as may be seen in Figure 3. The calf could stand and move around



FIG. 3. Calf born to cow 4E after about four months on the vitamin D deficient ration.

but its body was only about two-thirds as far from the ground as it should have been. A blood sample from the calf showed the calcium to be 11.46 mgm. and the inorganic phosphorus 7.10 mgm. per 100 cc. of plasma. In four days the legs of the calf had straightened considerably. The cow did not show any external symptoms of deficiency disturbances.

There was a partial recovery in the blood plasma calcium and inorganic phosphorus following the low points and fluctuations associated with the calving period, but in about six weeks the downward trend again prevailed. The milk flow was maintained at about 40 pounds daily during the flush of the lactation. The first mineral balance trial was started on January 26, 1937, eleven weeks after calving. The blood calcium and phosphorus were subnormal but stiffness had not developed. The negative balances shown in Table 1 for this trial indicate large drafts on the calcium and phosphorus reserves of the cow.

By the last of February, 15 weeks after parturition, stiffness was quite noticeable, and the animal was in a thin condition from being off-feed. The desired level of mineral intake was maintained, however, by increasing the allowance of bone meal supplement. By the time a second balance trial was completed the animal was very stiff and could scarcely get up and down, or move around. No vitamin D could be demonstrated in a sample of butterfat saved for assay at this time. The blood plasma calcium was down to 6.46 mgm. and the inorganic phosphorus to 1.34 mgm. per 100 cc. in the three-day sample taken just before viosterol feeding had to be started on March 16, about 18 weeks after parturition. Five cc. of viosterol were given daily. In four days she was eating better, and in seven days when the next balance trial was started her appetite was still better, her eyes brighter, and her ability to move about much improved although she was still noticeably stiff. According to the results of this trial as shown in Table 1, the negative balances had changed to strongly positive balances. In the fourth trial which followed immediately, the retention of calcium and phosphorus increased by another large increment. In the fifth and sixth trials coming at later intervals the large mineral retentions were well sustained. In all six of these trials the milk production remained quite constant and the mineral intake was fairly uniform at adequate normal levels. Five weeks after starting viosterol feeding the stiffness seemed to have disappeared and her appetite had improved so that she was fleshing up from the excess nutrients consumed. About the middle of June cod liver oil was substituted for the vitamin A concentrate and viosterol to supply vitamins A and D. On July 16, 1937, she showed estrum for the first time since calving on November 4, 1936. She had now recovered and was seemingly in a fair state of health.

The calcium and phosphorus content of the milk during this lactation is compared with similar data for a previous normal lactation in Charts 2 and 3. Again, the curves are quite remarkable for their similarity. Furthermore, the analysis of the milk from balance trials run before and after viosterol feeding show no consistent differences to indicate a decrease in the calcium and phosphorus content of milk produced under conditions of severe vitamin D deficiency. In the two trials before feeding vitamin D the calcium in the milk was 0.1266 per cent and 0.1126 per cent and the phosphorus 0.0995 per

cent and 0.0958 per cent respectively, while in the two trials run immediately after vitamin D administration the calcium was 0.1088 per cent and 0.1123 per cent and the phosphorus 0.0984 per cent and 0.0954 per cent respectively.

*Cow 1E.*—A few observations made on this animal will be presented chiefly as they concern the effects of a vitamin D deficiency on the developing fetus. This cow was on the vitamin D deficient ration and was also dry at the time she became pregnant. She was continued under these conditions throughout the gestation period. The blood plasma calcium and inorganic phosphorus remained normal until about six weeks or two months before parturition when a downward trend developed. A low of 8.50 mgm. of calcium and 3.08 mgm. of inorganic phosphorus per 100 cc. of plasma was reached. There were fluctuations at calving time followed by a temporary recovery, then a decline as the lactation proceeded.

Some idea of the condition of the calf may be obtained from the picture shown in Figure 4. The legs were really less useful than the picture would indicate as they would bend and twist into most any shape if the calf tried to move about. The calcium in the blood plasma of the calf was 11.44 mgm. and the inorganic phosphorus 5.81 mgm. per 100 cc. The calf seemed to im-

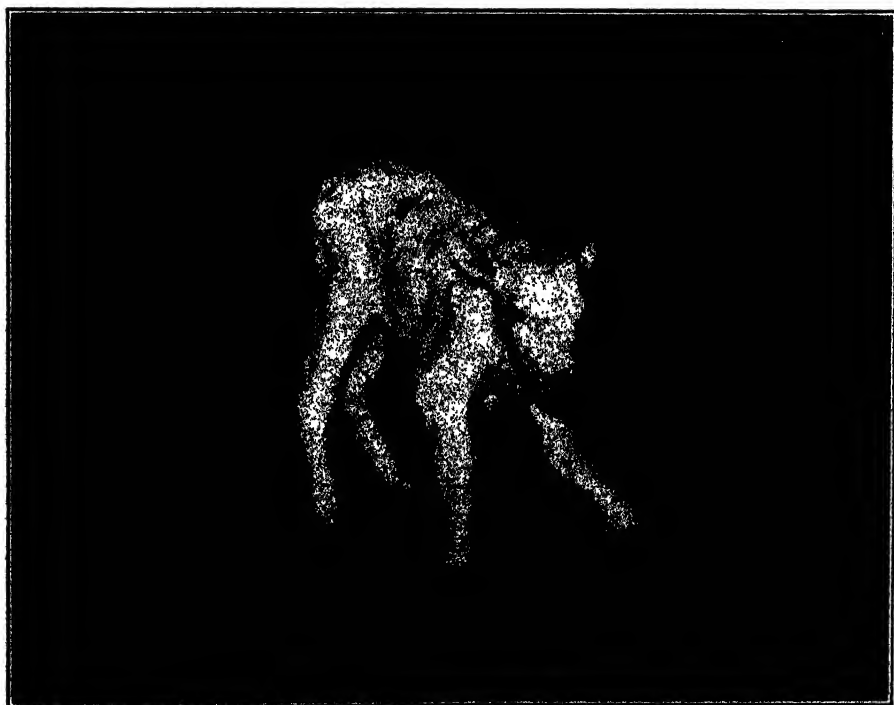


FIG. 4. Calf from cow 1E. This cow was dry and on the vitamin D deficient ration throughout the entire gestation period.

prove slightly for 3 or 4 days then appeared to grow weaker again. It developed a light case of scours and died on the seventh day. It was fed only the milk from its vitamin D deficient mother. There was a feeling that the calf would have stood a good chance to live had it received some cod liver oil or other source of vitamin D, and possibly some vitamin A. The costochondral junctions of the ribs seemed to be enlarged so a histological study was made on the seventh rib by Dr. J. B. Taylor of the Veterinary Department. The structure was found to be essentially normal with regular columns of cartilage cells and a smooth line at the junction between the cartilage and calcified area. The ribs seemed light in weight and had only a very thin shell of mineral deposit. A little pressure caused the left sixth rib to crease and bend as a hollow straw. Analysis showed that on the green weight basis this bone contained 66.06 per cent moisture, 14.28 per cent ash, and 0.34 per cent fat (this determination may be a little low), leaving 19.32 per cent for other organic materials. The ash contained 38.24 per cent of calcium and 19.75 per cent of phosphorus.

#### DISCUSSION

In this section the data and observations just presented for individual animals will be analyzed for the broader implications and generalizations which may be drawn to suggest expected or possible effects of a vitamin D deficiency on mature dairy cows.

#### *Total Calcium and Inorganic Phosphorus of the Blood Plasma*

By referring to Chart 1 it may be noted that a gradual decline in the total calcium and the inorganic phosphorus content of the blood plasma set in soon after the cows were placed under vitamin D deficient conditions. Six weeks or so before calving the rate of decline was accelerated presumably because of increased demands on the mother made by the rapidly developing fetus. At calving time there were fluctuations up and down but usually all at subnormal levels. Following the stress of the parturition period there may be a partial recovery to normal concentrations but the requirements for a liberal milk flow initiated a downward trend again in two or three months. In about six months the level of calcium had reached a low of from 5-7 mgm. per 100 cc. of plasma in some cases, and the inorganic phosphorus had declined to 1-2 mgm. Upon the administration of viosterol to supply vitamin D recovery was rapid so that in about two or three weeks the concentrations were well back toward, or within, the normal range.

These observations were made on cows due to freshen within a few months of the time the experiment was started, and they were also liberal milk producers. Whether or not non-pregnant, dry cows and poor producing animals will respond similarly are unanswerable questions which are under investigation at the present time.

*The Physical Condition of the Animal*

With the exception of the peculiar paralysis of 3E at the time of parturition, these three heavy-milking cows all began to develop stiffness under vitamin D deficiency conditions about three or four months after the beginning of the lactation. It usually developed shortly after the decline in blood plasma calcium and phosphorus had been definitely established. From a slight stiffness the condition grew gradually more severe. The knees bent forward, the ankles straightened throwing the weight forward onto the toes, the joints showed some swelling, the back became slightly arched and quite rigid so that it was not flexed in walking, turning, or in other body movements. The gait was stiff, slow, and shuffling, when the animal was forced to move. The appetite usually failed some, and in two or three months the condition was often so severe that assistance was necessary in getting the animal to its feet. After viosterol administration to supply vitamin D some improvement in the appetite and ease of body movements could usually be noted in about ten days. Gradual improvement continued so that within one or two months the animal was apparently normal again as far as could be seen from external observations. Whether these conditions would develop with cows milking less liberally or with dry cows are questions under investigation at the present time.

*The Calcium and Phosphorus Balances*

The results of ten-day mineral balance trials distributed at advantageous points over the period of observation on these cows are summarized in Table 1. The trials run while the vitamin D deficiency was developing show negative balances for calcium in all cases and for phosphorus in all but two cases for which approximate equilibrium is indicated. When the mineral feeding was continued at a level adequate for maintenance and the amount of milk produced, these negative balances were all changed to strong positive balances upon the administration of viosterol to supply vitamin D. The data for 4E are especially pertinent for in this case the milk production remained practically constant for six different trials and the mineral intake quite uniform and never fell to questionable levels at any time through loss of appetite or other causes. The two trials run under vitamin D deficient conditions show significant losses of minerals from the body. Immediately upon administration of vitamin D these losses were converted into large retentions amounting to nearly one-half pound of calcium and one-fourth pound of phosphorus in a ten-day period. It is recognized that heavy-milking cows in the flush of the lactation often show negative calcium and phosphorus balances for a short time but positive balances have usually been reestablished before the milk production has declined to the levels indicated in these trials. That vitamin D plays an important role in the ability of heavy-milking cows to utilize calcium and phosphorus is indicated by the unusual losses of these min-

erals under vitamin D deficient conditions and the abrupt change to large retentions upon the administration of viosterol. It may be noted from the total figures at the bottom of Table 1, that the losses from the body during vitamin D deficiency and the subsequent gains following viosterol administration were both in approximately a 2 to 1 ratio and suggest a depletion followed by a subsequent restoration of the mineral reserves of the skeleton.

On first thought it might appear that these results are at variance with those previously referred to from the Wisconsin Station but the explanation undoubtedly lies in the two different approaches used. In the Wisconsin experiment vitamin D supplements were added to normal rations which undoubtedly carried enough of this factor to meet normal requirements so that no measurable effects on the calcium and phosphorus balances or condition of the animals were produced by adding still larger quantities of vitamin D. In the present work, the animals were first deprived of vitamin D whereupon deficiency symptoms developed thus providing favorable conditions for fundamental studies on the relation of vitamin D to the utilization of calcium and phosphorus as shown by balance trials, blood chemistry, and other conditions.

It is interesting to note that the effects of a vitamin D deficiency on the blood chemistry, physical condition, and calcium and phosphorus balances of mature heavy-milking cows as just related are very similar to those exhibited by young growing calves as reported by Bechdel, Landsburg, and Hill (1), Rupel, Bohstedt, and Hart (16), Duncan and Huffman (3), Gullickson, Palmer, and Boyd (5), and Wallis, Palmer, and Gullickson (18).

#### *Calcium and Phosphorus in the Milk*

The data obtained from analyzing two-day composite samples of milk from each cow at monthly intervals for calcium and phosphorus are shown graphically in Charts 2 and 3. In comparing them with results obtained in a previous normal lactation the similarity of the curves is the most striking feature. There is even a slight tendency for both the calcium and phosphorus concentration to be higher in the vitamin D deficient lactation. These curves are remarkably smooth, follow the normal curves closely, and show no tendencies to decline under these extreme conditions of vitamin D deficiency nor to rise upon the administration of vitamin D. There was, however, a more rapid fall in the amount of milk produced as the vitamin D deficiency became pronounced than was shown in normal lactations. It should be noted that none of the lactations of the vitamin D deficient period were entirely completed so the last part of these curves is not necessarily characteristic of completed lactation curves.

The data for the percentage of calcium and phosphorus in the milk from balance trials run before and after vitamin D therapy also support the same

conclusion; namely, that the vitamin D deficiency had no measurable influence on the concentration of calcium and phosphorus in the milk.

### *Vitamin D in Butterfat*

The data obtained in the assays indicate that in none of these samples of fat taken from the cows while suffering severely from a deficiency of vitamin D was it possible to demonstrate the presence of measurable amounts of vitamin D. Twelve grams of fat were mixed with the rachitogenic diet and fed during the first eight days of the test period. This is about all the rats would consume regularly during the test period so larger amounts could not be used. Unsuccessful attempts to concentrate any possible vitamin D in the butterfat so a more searching test could be made have been discussed by the author (17) on a previous occasion.

### *Effects on the Developing Fetus*

Cow 2E dropped a fine normal-appearing calf after two months of the dry period under the vitamin D deficient conditions. The calf from 3E born four months after starting the experiment, showed slightly bent knees and cocked-ankles, but straightened up in a week or so. The calf from 4E, born after four months on the deficient diet, is shown in Figure 3. The legs are extremely crooked, but the blood plasma calcium and inorganic phosphorus were normal. The legs gradually straightened over a period of two or three weeks. The calf from 1E is shown in Figure 4. This cow was dry and on the vitamin D deficient regime throughout the gestation period. The blood picture was essentially normal, and the histological studies made on the costochondral junction of the seventh rib after its death on the seventh day showed no evidences of rachitic malformations. The high moisture content and the low fat and ash contents indicated by the analyses of the sixth rib are interesting but at the present time we do not have figures from comparable normal calves with which to compare them. The calcium and phosphorus found in the ash approximate the figures generally reported for bone ash. The evidence indicates that calves born to cows maintained for a considerable length of time under vitamin D deficient conditions may have a decided rachitic appearance and possibly a lowered mineral content of the bones. Blood chemistry and histological studies, however, have shown normal conditions to prevail in these respects.

### *Bones of the Animal*

In connection with 2E the evidence has already been presented which may possibly indicate that the vitamin D deficiency has had some relation to the marked fragility of the bones encountered in this case.

### *Breeding Efficiency*

The breeding records are available for these animals for at least two lactations previous to the experiment. The regularity shown by these records stands out in striking contrast to the fact that none of these cows showed estrum during the period of vitamin D deficiency. It is not clear, however, whether this condition should be attributed to the lack of vitamin D *per se*, to the decline in general health and vigor of the animals, or perhaps to some other factor entirely.

### SUMMARY

Detailed observations on three liberal-milking cows and one dry, pregnant cow kept under vitamin D deficient conditions have been presented. Under these conditions the total calcium of the blood plasma declined to one-half normal values, and the inorganic phosphorus to one-fifth normal. The animals became stiff, the knees bent forward, the spine became rigid and in severe cases assistance was necessary before the cow could get up. Balance trials run while these conditions prevailed showed that significant drafts on the calcium and phosphorus reserves were being made. When vitamin D was administered the losses were immediately changed to unusually large retentions. The curves showing the calcium and phosphorus concentration in the milk are remarkably smooth and coincide closely with similar curves for previous normal lactations. Calves produced after the cows had been under the deficiency conditions for some time showed visible rachitic appearances but blood chemistry and histological studies revealed no abnormalities in these respects. Vitamin D could not be detected in butterfat samples saved from animals deficient in this factor. Whether or not the fragile bones encountered in one animal and the failure of all the animals to show estrum are directly connected with the lack of vitamin D cannot be established at the present time.

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## A STUDY OF METHODS FOR THE DETERMINATION OF ACIDITY IN BUTTER FAT\*

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During the past three years, a study of butter made from neutralized cream has received considerable attention in this laboratory. In connection with this project it seemed possible that valuable information might be obtained by determining the free acidity in the fat phase of the butter. The method employed is based on the general method given by Leathes (3). In addition, the details of the Leathes method for the quantitative separation of free acids from butter fat have been worked out because it is desirable at times to obtain these acids free from fat for further study. The following experiments were undertaken in order to check these methods quantitatively and to compare them with the Official method of the A. O. A. C. (1) and the recently described method of Clarke *et al.* (2).

### EXPERIMENTAL

**Reagents and apparatus.** Petroleum ether was distilled over solid KOH and the fraction which boiled between 30° and 40° C. was used. Benzene was likewise distilled from solid KOH. Absolute alcohol was refluxed for 1 to 2 hours over solid KOH and aluminum metal (10 gm. of each per liter) and then distilled from these reagents (6). Alcohol so purified was used in the preparation of 0.5 N KOH and  $C_2H_5ONa$  solutions. Indicator solutions were prepared as follows: 1 gm. of phenolphthalein was dissolved in 100 ml. of absolute alcohol after which alcoholic KOH was added until a faint pink color was produced. Ordinary 95 per cent alcohol was made very slightly alkaline to phenolphthalein with 0.1 N NaOH for the A. O. A. C. method.

Each of the alkaline solutions was protected from  $CO_2$  to minimize changes in concentration. A siphon from the reservoir was connected to a side arm at the base of the burette; the top of the burette was connected to a T-tube which extended just through the stopper into the reservoir, while a soda-lime tube was joined to the other arm of the T-tube. The alkaline solutions were

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standardized against 0.01 N HCl at frequent intervals; the greatest change in normality in a three month period was 0.0003. The HCl solution was standardized gravimetrically by precipitating and weighing the chlorine as AgCl.

All samples of fat for titration were weighed on a Torsion butter moisture balance. The sensitivity of this balance was such that 0.02 gm. displaced the zero reading 0.5 scale space. It is therefore considered that the samples could be weighed to  $\pm 0.01$  gm. of the desired weight. In all cases this would represent an amount of acid too small to be of any significance in the titrations.

The acids and fat (in the preparation of fat-acid mixtures) and the extracted acids were weighed on an Ainsworth type TC balance. The weighings were estimated to 0.01 mg. by the method of swings.

An International size 2 centrifuge was used in the separation of acids from fat.

**Methods.** The acidity determinations were made on two types of materials. In one type, the acids were removed as completely as possible from a large sample (about 1000 gm.) of high quality butter fat and definite mixtures by weight of this fat and various fatty acids were prepared; in the second, the filtered fats from samples of market butter secured from several sources were employed.

The acid-free fat was prepared as follows: The butter was heated in a water bath at  $55^{\circ}$  to  $60^{\circ}$  C. until a clear fat sample was obtained when filtered. Lots of about 130 gm. of this fat were weighed into 150 ml. beakers, transferred to 1 liter separatory funnels with 400 ml. of petroleum ether. Then 160 ml. of absolute alcohol and 10 drops of phenolphthalein were added and the acids were neutralized with 1 N alcoholic KOH. In order to minimize hydrolysis of the soaps formed, 10 ml. of  $\text{NH}_4\text{OH}$  (28 per cent  $\text{NH}_3$ ) were added and mixed with the solution; 150 ml. of  $\text{H}_2\text{O}$  were added next and the contents were mixed by rotating for 2 minutes. When separation appeared to be complete, the lower layer containing the soaps was removed. One hundred milliliters of absolute alcohol were added to and mixed with the fat-ether solution, after which 100 ml. of  $\text{H}_2\text{O}$  were added, the funnels were shaken vigorously for 2 minutes and the aqueous-alcoholic layer was again removed. This process was repeated using 50 ml. each of absolute alcohol and water and was followed by two washings with 100 ml. of water alone. After the water was removed as completely as possible, the fat solutions were poured from the separatory funnels into large Erlenmeyer flasks and were dried for several hours with anhydrous  $\text{Na}_2\text{SO}_4$ . The solution was filtered and the petroleum ether was distilled from the fat; the last traces of petroleum ether were removed by heating in the oven of the Mojonnier tester at  $100^{\circ}$  C. and 20 in. vacuum for 30 minutes.

The saturated acids employed in these experiments were obtained from

Eastman Kodak Company and were used without further purification. The oleic acid was partially purified by crystallization of the lithium salt from 50 per cent alcohol (5) and was then distilled under reduced pressure. However, it is evident from the neutralization equivalent obtained that it was not pure.

The insoluble butter acids were prepared by boiling 10 gm. of butter fat and 75 ml. 1 N alcoholic KOH under reflux for 30 minutes, removing the reflux condenser, adding 200 ml. of H<sub>2</sub>O and continuing the heating until the alcohol had been removed. The soap solution was diluted to about 800 ml. with boiling water and was acidified with dilute H<sub>2</sub>SO<sub>4</sub>. This mixture was heated until the acids separated as an oily layer on the surface, after which the beaker and its contents were cooled until the solid cake of insoluble acids could be transferred to another beaker of boiling water. The alternate heating and solidification process was repeated until there was very little odor of the lower molecular weight acids. Moisture was removed from the solid cake of acids by pressing between layers of filter paper. The acids were next dissolved in petroleum ether and the excess water was removed in a separatory funnel. The solution was dried for several hours over Na<sub>2</sub>SO<sub>4</sub>, filtered, the bulk of the petroleum ether was evaporated and the last traces of it were removed in the vacuum oven at 100° C. and 20 in. vacuum for 30 minutes.

The methods employed to determine the amounts of free acids in the fat-acid mixtures and in the ordinary samples of butter fat are:

A. Official method of the A. O. A. C. (1). Twenty grams of fat were weighed into 125 ml. Erlenmeyer flasks, 50 ml. of neutralized alcohol and 10 drops of phenolphthalein were added, the contents were heated to boiling, the flasks were shaken vigorously and the titrations were made with 0.1 N NaOH. An attempt was made to obtain an end point which would persist for 30 seconds.

B. Method of Clarke *et al.* (2). Five grams of fat were weighed into 125 ml. Erlenmeyer flasks, 15 ml. of benzene and 5 drops of phenolphthalein were added and the titrations were made with 0.05 N C<sub>2</sub>H<sub>5</sub>ONa in absolute alcohol. Blank titrations on the benzene were subtracted from the burette readings of the samples.

C. Alcoholic KOH method (A). Ten grams of fat were weighed into 125 ml. Erlenmeyer flasks and dissolved in 25 ml. of petroleum ether; 10 ml. of absolute alcohol and 10 drops of phenolphthalein were added and the titrations were made with 0.05 N KOH in absolute alcohol. The first definite color change (compared with a sample which had not been titrated) was taken as the end-point. Blank determinations on the reagents were subtracted from the burette readings of the samples.

D. Alcoholic KOH method (B). Ten grams of fat were weighed into 50 ml. beakers, transferred to 150 ml. Squibb type separatory funnels (designed for use in the centrifuge) with 50 ml. of petroleum ether; 25 ml. of

absolute alcohol and 10 drops of phenolphthalein were added and the titrations were made as described in C. One milliliter of  $\text{NH}_4\text{OH}$  (28 per cent  $\text{NH}_3$ ) was added and mixed with the solution. An amount of water equal to the total amount of alcohol present was added, the funnels were stoppered, shaken vigorously for 2 minutes and were centrifuged at approximately 1000 revolutions per minute until the emulsion had been broken—usually 5 minutes was sufficient. The lower layers (solutions of soaps) were run into 250 ml. centrifuge bottles. Ten milliliters of absolute alcohol were added to and mixed with the fat solution, 10 ml. of  $\text{H}_2\text{O}$  were added and the funnels were again shaken vigorously and centrifuged. The washing of the fat was repeated using 5 ml. each of absolute alcohol and water. The combined soap solution and washings were twice shaken vigorously for about 2 minutes with 50 ml. portions of petroleum ether and were centrifuged at 1500 to 1800 revolutions per minute for 15 to 20 minutes to free them from fat. The petroleum ether layer was forced from the bottle through a small glass tube (fitted in a two-hole rubber stopper) by means of air pressure from an atomizer bulb. The petroleum ether was blown into a small separatory funnel and the small amounts of soap solution frequently included were returned to the centrifuge bottles. The soap solutions were acidified with 3 ml. of  $\text{H}_2\text{SO}_4$  (1 part  $\text{H}_2\text{SO}_4$  to 4 parts  $\text{H}_2\text{O}$  by volume). The solutions at this stage should be strongly acid to methyl orange. The free fatty acids were extracted as follows: The acidified solutions were shaken with three successive portions (50, 25, and 25 ml. respectively) of petroleum ether; after each treatment the flasks were centrifuged and the petroleum ether layer was drawn off. The combined extracts were washed with 50 ml.  $\text{H}_2\text{O}$  in 250 ml. separatory funnels to remove traces of  $\text{H}_2\text{SO}_4$ . These acid extracts were poured into weighed 125 ml. Erlenmeyer flasks, the petroleum ether was slowly evaporated on an electric hot plate under the hood and the last traces were removed in the vacuum oven at  $85^\circ$  to  $90^\circ$  C. and 20 in. vacuum for 15 to 20 minutes. After cooling the flasks were reweighed; the recovered acids were dissolved in 50 ml. of petroleum ether, 25 ml. of absolute alcohol and 10 drops of phenolphthalein were added and the titrations were made with 0.05 N alcoholic KOH solution. Blank determinations made on the solvents were subtracted from the samples.

The neutralization equivalents of the fatty acids used in these experiments were determined by the method of Sandin, Kulka and Woolney (4). The  $\text{C}_2\text{H}_5\text{ONa}$  solution was standardized against 0.01 N HCl rather than highly purified stearic acid as recommended by these authors. The calculated titration values were then obtained as follows:

$$\text{Calculated ml. 0.1 N alkali} = \frac{\text{Weight of acid} \times 10,000}{\text{Neutralization equivalent}}$$

**Results.** The analytical data obtained with the above methods are presented in Tables 1 and 2. The two alcoholic KOH methods, whose values are nearly identical, agree more closely with the calculated values than do

TABLE 1  
Acidity determinations of butterfat—fatty acid mixtures

Kind of acid added	1 Neut. equiv. of acids added to fat	Titration values: ml. 0.1 N alkali per 10 gm. sample						9 Weight of acid recovered	10 % of weight of acid added recovered	11 Titrat. recovered acids as % orig. titrat. col. $\frac{7}{6} \times 100$
		2 Calc. from neut. equiv. and weight of acid	3 A.O.A.C. method	4 Method of Clarke <i>et al.</i>	5 Alcoholic KOH method <sup>1</sup> (A)	6 Alcoholic KOH method <sup>2</sup> (B)	7 Recov. erd acids	8 Weight of acid added per 10 gm. sample		
None	a		0.10	0.02	0.05	0.05	0.01	0.00102		
	b		0.10	0.02	0.04	0.04	0.01	0.00112		22.2
	av.		0.100	0.020	0.045	0.045	0.010	0.00107		
Lauric	a		2.36	2.45	2.35	2.33	2.22	0.04615		
	b		2.38	2.42	2.34	2.34	2.25	0.04656		
	av.		2.370	2.435	2.345	2.335	2.235	0.04636		
Myristic	corr. <sup>3</sup>	2.290	2.270	2.415	2.300	2.290	2.225	0.04529	98.3	97.2
	a		2.25	2.34	2.24	2.23	2.16	0.05030		
	b		2.27	2.33	2.24	2.24	2.17	0.05019		
Palmitic	av.		2.260	2.335	2.240	2.235	2.165	0.05025		
	corr. <sup>3</sup>	2.218	2.160	2.315	2.195	2.190	2.155	0.04918	98.2	98.4
	a		1.96	2.04	1.94	1.95	1.87	0.04929		
Stearic	b		1.96	2.06	1.94	1.94	1.87	0.04937		
	av.		1.960	2.050	1.940	1.945	1.870	0.04933		
	corr. <sup>3</sup>	1.910	1.860	2.030	1.895	1.900	1.860	0.04826	98.8	97.9
Oleic	a		1.86	1.91	1.83	1.82	1.74	0.05103		
	b		1.85	1.90	1.83	1.84	1.75	0.05146		
	av.		1.855	1.905	1.830	1.830	1.745	0.05125		
Oleic	corr. <sup>3</sup>	1.790	1.755	1.885	1.785	1.785	1.735	0.05018	99.4	97.2
	a		1.90	1.94	1.86	1.85	1.76	0.05114		
	b		1.89	1.93	1.86	1.85	1.76	0.05129		
Oleic	av.		1.895	1.935	1.860	1.850	1.760	0.05122		
	corr. <sup>3</sup>	1.815	1.795	1.915	1.815	1.805	1.750	0.05015	97.0	
								0.05172		

TABLE 1—(Continued)

Kind of acid added	1 Neut. equiv. of acids added to fat	Titration values: ml. 0.1 N alkali per 10 gm. sample						8 Weight of acid added per 10 gm. sample	9 Weight of acid recovered	10 % of weight of added acid recovered	11 Titrat. recovered acids as % orig. titrat. col. $\frac{7}{6} \times 100$
		2 Calc. from neut. equiv. and weight of acid	3 A.O.A.C. method	4 Method of Clarke <i>et al.</i>	5 Alcoholic KOH method <sup>1</sup> (A)	6 Alcoholic KOH method <sup>2</sup> (B)	7 Recover. acids				
Insoluble butter acids	a		0.51	0.50	0.45	0.45	0.38		0.01130		
	b		0.52	0.50	0.46	0.45	0.38		0.01117		
	av. corr. <sup>3</sup>	0.400	0.515	0.500	0.455	0.450	0.380	0.01075	0.01124	94.6	91.4
Insoluble butter acids	a		1.96	1.96	1.87	1.87	1.76		0.01017		
	b		1.96	1.95	1.87	1.87	1.76		0.04836		
	av. corr. <sup>3</sup>	1.827	1.960	1.955	1.870	1.870	1.760		0.04817		
Insoluble butter acids	a		1.860	1.935	1.825	1.825	1.750	0.04909	0.04827	96.2	95.9
	b		3.86	3.90	3.79	3.80	3.63		0.04720		
	av. corr. <sup>3</sup>	3.764	3.84	3.91	3.80	3.80	3.60		0.09894		
			3.850	3.905	3.795	3.800	3.615		0.09839		
			3.750	3.885	3.750	3.755	3.605	0.10115	0.09867	96.5	96.0
									0.09760		

<sup>1</sup> 10 gm. sample weighed into 125 ml. Erlenmeyer flask, dissolved in 25 ml. petroleum ether, 10 ml. absolute alcohol added and titrated with KOH in absolute alcohol.

<sup>2</sup> 10 gm. sample weighed into 50 ml. beaker, transferred to 150 ml. separatory funnel with 50 ml. petroleum ether, 25 ml. absolute alcohol added and titrated with KOH in absolute alcohol.

<sup>3</sup> The average value of the sample containing no added acid was subtracted from the averages of the other samples to obtain the corrected values.

TABLE 2

*Acidity determinations of butterfat from samples of commercial butter*

Sample number	Titration values: ml. 0.1 N alkali per 10 gm. fat					6 Weight of re-covered acids	7 Titrat. recovered acids as % orig. titrat. $\frac{\text{col. 5}}{\text{col. 4}} \times 100$
	1 A.O.A.C.	2 Method of Clarke <i>et al.</i>	3 Alcoholic KOH method <sup>1</sup> (A)	4 Alcoholic KOH method <sup>2</sup> (B)	5 Re-covered acids		
1	a	0.50	0.59	0.56	0.56	0.51	0.01392
	b	0.51	0.60	0.56	0.55	0.52	0.01375
	av.	0.505	0.595	0.560	0.555	0.515	0.01384
2	a	0.75	0.78	0.72	0.73	0.69	0.01863
	b	0.76	0.78	0.73	0.73	0.69	0.01859
	av.	0.755	0.780	0.725	0.730	0.690	0.01862
3	a	1.34	1.42	1.34	1.36	1.28	0.03473
	b	1.34	1.43	1.34	1.34	1.28	0.03470
	av.	1.340	1.425	1.340	1.350	1.280	0.03472
4	a	0.71	0.78	0.72	0.71	0.67	0.01814
	b	0.71	0.78	0.71	0.72	0.67	0.01817
	av.	0.710	0.780	0.715	0.715	0.670	0.01816
5	a	0.74	0.80	0.74	0.76	0.72	0.01916
	b	0.73	0.80	0.74	0.74	0.72	0.01936
	av.	0.735	0.800	0.740	0.750	0.720	0.01926
6	a	0.74	0.79	0.72	0.74	0.67	0.01805
	b	0.74	0.78	0.72	0.74	0.67	0.01814
	av.	0.740	0.785	0.720	0.740	0.670	0.01810
7	a	0.59	0.66	0.61	0.64	0.57	0.01526
	b	0.57	0.68	0.61	0.62	0.56	0.01515
	av.	0.580	0.670	0.610	0.630	0.565	0.01522
8	a	0.56	0.64	0.59	0.59	0.55	0.01564
	b	0.56	0.64	0.59	0.60	0.55	0.01503
	av.	0.560	0.640	0.590	0.595	0.550	0.01534
9	a	0.70	0.71	0.71	0.67	0.63	0.01720
	b	0.75	0.71	0.70	0.68	0.64	0.01710
	av.	0.725	0.710	0.705	0.675	0.635	0.01715
10	a	0.60	0.65	0.64	0.64	0.58	0.01597
	b	0.59	0.65	0.64	0.63	0.58	0.01556
	av.	0.595	0.650	0.640	0.635	0.580	0.01577
11	a	0.91	0.86	0.81	0.80	0.75	0.02058
	b	0.86	0.86	0.80	0.81	0.75	0.02054
	av.	0.885	0.860	0.805	0.805	0.750	0.02056
12	a	0.63	0.65	0.62	0.62	0.56	0.01566
	b	0.70	0.67	0.61	0.62	0.55	0.01524
	av.	0.665	0.660	0.615	0.620	0.555	0.01545
13	a	0.79	0.78	0.72	0.72	0.66	0.01818
	b	0.77	0.76	0.71	0.72	0.66	0.01821
	av.	0.780	0.770	0.715	0.720	0.660	0.01820
14	a	0.75	0.78	0.75	0.76	0.70	0.01888
	b	0.79	0.78	0.75	0.75	0.70	0.01869
	av.	0.770	0.780	0.750	0.755	0.700	0.01879
15	a	0.47	0.50	0.48	0.50	0.43	0.01222
	b	0.44	0.52	0.47	0.50	0.43	0.01192
	av.	0.455	0.510	0.475	0.500	0.430	0.01207
16	a	1.21	1.33	1.23	1.26	1.14	0.03031
	b	1.18	1.34	1.24	1.26	1.14	0.03052
	av.	1.195	1.335	1.235	1.260	1.140	0.03042

TABLE 2—(Continued)

Sample number	Titration values: ml. 0.1 N alkali per 10 gm. fat					6 Weight of re-covered acids	7 Titrat. recovered acids as % orig. titrat. col. 5 col. 4 $\times 100$
	1 A.O.A.C.	2 Method of Clarke <i>et al.</i>	3 Alcoholic KOH method <sup>1</sup> (A)	4 Alcoholic KOH method <sup>2</sup> (B)	5 Re-covered acids		
17	a	0.79	0.89	0.84	0.84	0.78	93.5
	b	0.84	0.91	0.85	0.84	0.79	
	av.	0.815	0.900	0.845	0.840	0.785	
18	a	1.65	1.76	1.67	1.67	1.59	94.8
	b	1.63	1.76	1.67	1.66	1.60	
	av.	1.640	1.760	1.670	1.665	1.595	
19	a	1.17	1.27	1.18	1.18	1.10	93.6
	b	1.14	1.25	1.18	1.17	1.10	
	av.	1.155	1.260	1.180	1.175	1.100	
20	a	1.38	1.50	1.40	1.41	1.34	95.4
	b	1.38	1.48	1.41	1.42	1.36	
	av.	1.380	1.490	1.405	1.415	1.350	
21	a	0.65	0.71	0.66	0.67	0.58	85.8
	b	0.66	0.70	0.67	0.67	0.57	
	av.	0.655	0.705	0.665	0.670	0.575	
Averages	0.840	0.898	0.843	0.848	0.787	0.02137	92.1

<sup>1</sup> See table 1.<sup>2</sup> See table 1.

the methods of Clarke *et al.* and the A. O. A. C. The method of Clarke *et al.* gives somewhat higher values than the calculated while the A. O. A. C. method gives slightly lower values. If these two methods are compared with the alcoholic KOH methods in Table 2, the same relationships hold among the average values. If the average values (uncorrected) from Table 1 are compared, it will be observed that the alcoholic-KOH method still yield values that agree more closely with the calculated values than do those of the other methods. It should be emphasized, however, that the differences between these methods are not very great and probably have little significance—at least for comparative work.

Considerable difficulty was encountered in obtaining good checks between duplicate determinations with the A. O. A. C. method as a result of the rapidly fading end-point. With the method of Clarke *et al.* the end-points were somewhat obscured in the samples containing the pure saturated fatty acids because the soaps formed in the titrations precipitated to a considerable extent. This did not occur appreciably with the other samples in Table 1 or with the samples of ordinary butter fat. It is believed that the alcoholic KOH method (A) offers some advantages over the other two methods in that the end-points persist longer, the color changes are more easily observed, and somewhat better agreement between duplicate determinations is obtained.

No attempt has been made to identify the material extracted in the control sample. Since the method of removing the acids originally present in the

fat so closely resembles the method employed in obtaining the data of Table 1, it is difficult to explain the presence of the acids in the control unless a small amount of hydrolysis of fat occurred after the removal of the original acids. It is possible that this extracted material may contain some fat, phospholipin or both.

The percentages of acids recovered with the extraction method are given in columns 10 and 11 of Table 1 and column 7 of Table 2. The most probable explanations for the 22.2 per cent recovery of the titratable acidity in the control sample are: (a) that more alkali was required to produce a perceptible end-point in the presence of the fat than in a clear solution, and (b) that the fat contained a small amount of volatile acidity which was lost during the extraction process. Table 1 shows that the titratable acidity of the recovered acids (column 7) as percentage of the original titration (column 6) agrees rather well with the percentage of the weight of added acids which was recovered. In Table 2 the titratable acidities of the recovered acids are lower percentage values (column 7) of the original titrations than are the corresponding values of Table 1. No control could be employed here as was done with the values in Table 1; it is logical to assume, therefore, that error resulting from the masking of end-points is largely responsible for the lower values obtained when the titration of the recovered acids is calculated as percentage of the titration in the presence of fat. The samples used for the values of Table 2 were more highly colored than those in Table 1. Volatile acids in the fat may account for a part of the discrepancy.

Some information regarding the purity of the extracted acids can be obtained by calculating their neutralization equivalents from the data given in column 7 and 9 of Table 1 and comparing them with the values given in column 1. These calculations indicate that the acids are at least 97 per cent of their original degree of purity.

#### SUMMARY AND CONCLUSIONS

1. Methods were described for the titration of free acids in butter fat and for the separation of these acids from the fat. Comparisons of these methods with the A. O. A. C. method and that of Clarke *et al.* were presented for butter fat samples from commercial butters and for samples of fat to which definite weights of fatty acid were added.

2. Good agreement was obtained among the titration methods although the method of Clarke *et al.* gave slightly higher values than the other methods.

3. The titration end-points were most easily observed with the alcoholic KOH methods and the best agreement between duplicates was obtained with these methods. The end-points of the A. O. A. C. method were the most difficult and for this reason the duplicates did not agree as closely as with the other methods.

4. From 94.6 to 99.4 per cent of the weight of added acids were recovered by the acid separation method described. Apparently these acids were at least 97 per cent of their original degree of purity.

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# THE EFFECT OF FEEDING MANGELS OR DRIED BEET PULP TO COWS ON THE DEVELOPMENT OF OXIDIZED FLAVOR IN MILK\*

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The milk of the Experiment Station Jersey herd has been unusually free from oxidized flavors in comparison with milk from other local sources. This herd, for many years, has been fed mangels during the winter months which is contrary to the usual feeding practice.

It is well known that cows on pasture or cows fed green cut legumes or grasses produce milk which is less susceptible to the development of oxidized flavors. Likewise, it has been shown by Anderson<sup>1</sup> that the feeding of carrots during the winter months tends to prevent the development of this flavor in milk. In view of these results and since feed is one of the many variable factors in the development of this flavor it is logical to assume that the feeding of mangels might be a factor in preventing its development.

Dried beet pulp has never been fed to our herd but is very common in dairy rations. Since Davies<sup>2</sup> reported that the feeding of dried beet pulp sometimes caused a fishy or off-flavor in milk it seemed advisable to ascertain whether or not this flavor might be related to an oxidized flavor.

## EXPERIMENTAL PROCEDURE

As soon as the station Jersey herd of 26 milking cows was removed from pasture in the fall weekly samples were taken of approximately one quart of milk from each cow at the noon milking. One pint bottle of each of these samples was pasteurized at a temperature of 143° F. to 145° F. for one-half hour in a water bath. After pasteurization half of each pint of hot milk was poured into half pint bottles containing sufficient copper sulphate solution to increase the copper content by 0.25 p.p.m. It was desirable to add copper to part of the milk to determine more accurately the degree of susceptibility of the milk from each cow to the production of oxidized flavors. The milk was all cooled in the bottles in an ice water bath. The raw milk was judged for flavor by the authors shortly after milking and the raw, pasteurized and pasteurized plus copper milks were judged the first, third, and fifth days after milking and pasteurization.

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<sup>1</sup> The Cause of Rancid and Oxidized Flavors in Raw Milk. J. A. Anderson, Proceedings International Association of Milk Dealers, Lab. Section, pp. 117-134, 1937.

<sup>2</sup> The Fishy Flavor of Milk Caused by Feeding Beet By-products. W. L. Davies, Agricultural Progress 13, 112-5, 1936.

TABLE 1  
Average of three weeks' scores of milk when no mangels were fed

Cow No.	Fresh			1 day old			3 days old			5 days old		
	Raw	Past.	Past. plus .25 p.p.m. copper	Raw	Past.	Past. plus .25 p.p.m. copper	Raw	Past.	Past. plus .25 p.p.m. copper	Raw	Past.	Past. plus .25 p.p.m. copper
1	23†	22‡	23‡	22‡	22‡	17‡*	22‡	22‡	20‡*	22‡	20‡*	17 *
2	23‡	22‡	23‡	22‡	22‡	17‡*	22‡	22‡	22‡	22‡	22‡	17 *
3	23‡	22‡	23‡	22‡	22‡	20 *	23	22‡	22‡	22‡	22‡	19‡*
4	23	22‡	22‡	22‡	22‡	17‡*	22‡	21‡*	19 *	21‡	19 *	17‡*
5	23	22‡	22‡	22‡	22‡	20‡*	22‡	22‡	22	22‡	22	20
6	23‡	22‡	22‡	22‡	22‡	15‡*	22	22‡	21‡*	20‡	21‡*	17‡*
7	23‡	22‡	22‡	22‡	22‡	16‡*	22‡	22‡	19‡	20‡	19‡	17‡*
8	23‡	22‡	22‡	22‡	22‡	22	22‡	22‡	22	22‡	22	18 *
9	23‡	22‡	22‡	22‡	22‡	19 *	23	22‡	21 †	22‡	21 †	18‡*†
10	23‡	22‡	22‡	22‡	22‡	18‡†	22‡	22‡	22	22‡	22	18‡*
11	23‡	22‡	22‡	22‡	22‡	19‡*	22‡	22‡	21‡	22‡	21‡	18 *
12	23‡	22‡	22‡	22‡	22‡	18‡*	22‡	22‡	21‡	22‡	21‡	18 *
13	23‡	22‡	22‡	22‡	22‡	21 *	22‡	22‡	21‡	22‡	21‡	19 *
14	23‡	22‡	22‡	22‡	22‡	17‡*†	22‡	22‡	19‡*	22‡	19‡*	18‡*†
15	23‡	21 †	20‡†	21 †	20‡†	17‡*	21	20‡†	21 *	20‡†	21 *	20 *
16	23‡	21 †	20‡†	21	20‡†	17‡*	22	21‡	21 *	21‡	21 *	18
17	23	22‡	22 *	22	21‡	19‡*†	22	21‡	21 *	21‡	21 *	18
18	23	22‡	21‡†	22‡	21‡†	16 †	22‡	22	19‡*	21‡	19‡*	18
19	23‡	22‡	21‡*	22‡	22	21 *	22‡	22	22	22	22	19 *
20	23‡	22‡	22	22‡	22‡	17‡*	22‡	22‡	22‡	22‡	22‡	20‡*
21	23	22‡	22	22‡	22‡	19‡*	22‡	22‡	22	22	22	19 *
22	23	22‡	22	22‡	22‡	19‡*	22‡	22‡	22‡	22‡	22‡	20‡*
23	23	22‡	22	22‡	22‡	19‡*	22‡	22‡	22‡	22‡	22‡	20‡*
24	23‡	22‡	22‡	22‡	22‡	19‡*	22‡	22‡	22‡	22‡	22‡	20‡*
25	23‡	22‡	22‡	22‡	22‡	19‡*	22‡	22‡	22‡	22‡	22‡	20‡*
26	23‡	22‡	22‡	22‡	22‡	19‡*	22‡	22‡	22‡	22	22‡	20‡*

\* oxidized, † salty. All other cuts for old or heated flavors.

The first three weeks after the cows were off pasture none of the herd received mangels.

## RESULTS

### *The Effect of Feeding Mangels*

The milk from each cow was handled and scored for flavor as described in the experimental procedure. Table 1 gives the average score of the milk for three weeks when no mangels were fed. No oxidized flavor developed in any of the samples of raw milk even after five days at 40° F. Only one sample of pasteurized milk developed an oxidized flavor after three days and four samples after five days. The milk from cow number five was most susceptible to the development of oxidized flavors and after the cows had been off pasture for a longer period the raw milk from this cow became oxidized after five days. As shown in table 1 only four of the samples to which copper had been added became oxidized after one day but practically all of them became oxidized on the third and fifth day.

Beginning the following week the cows number 1 to 13, inclusive, were fed mangels. There was no selection in the group and they constitute one row of cows in the barn. The milk from the individual cows and a composite sample of the milk from cows number 1 to 13 and cows 14 to 26 were handled as previously described. The average scores for this three weeks period are given in Table 2. As can be seen from this table the feeding of mangels apparently exerted no influence in preventing the development of oxidized flavors. Likewise the composite samples of the mangel and non-mangel group in Table 2 showed no difference in their susceptibility to the development of oxidized flavors.

Since the first two tables are averages of scores for three week periods they do not give the frequency of occurrence of oxidized flavor for a given number of samples tested. Table 3 gives this information for the two periods of three weeks. In the first three weeks tests the samples containing copper were tasted on the fifth day only during the last week and the first weeks samples did not have copper added to them.

The first half of Table 3 gives the occurrence of oxidized flavor when no mangels were fed. The cows in this table are nevertheless divided into groups 1 to 13 and 14 to 26. The group from 1 to 13 was selected to be fed mangels since the oxidized flavor occurred more often in this group and should mangels help to prevent the development of this flavor it would be more noticeable in this group.

The second part of Table 3 shows the occurrence of oxidized flavors in the same two groups when cows 1 to 13 are fed mangels. The raw milk of cow number 5 developed an oxidized flavor at this time after five days holding and is responsible for the occurrence of this flavor as shown in the table. Table 3 might indicate that the milk from the group receiving mangels was

TABLE 2  
Average of three weeks' scores of milk when half the herd received no mangels and half received mangels

Cow No.	Fresh		1 day old		3 days old			5 days old		
	Raw	Past.	Past.	Past. plus .25 p.p.m. copper	Raw	Past.	Past. plus .25 p.p.m. copper	Raw	Past.	Past. plus .25 p.p.m. copper
Mangels										
1	23†	23	23	23	22†	22†	19‡*	21‡	21‡	15‡*
2	23†	23	23	23	22†	22†	16‡*	21‡	21‡	15‡*
4	23	23	23	23	22†	22†	19 *	21‡	21	17‡*
5	23†	23	23	31‡*	23†	20‡*	17‡*	20‡*	17‡*	15 *
6	23†	23	23	23	23†	23†	21‡*	21‡	21‡†	18‡*
8	22††	22††	22††	21 †	21††	20‡*†	17‡*†	20‡*†	18 *	16‡*
9	23†	23	23	23	23†	23†	21 *	21‡	21‡	17‡*
10	23	22††	22††	22††	22 †	22††	20‡*	20‡†	20‡†	17‡*
11	23†	23	23	23	22†	22†	17 *	21‡	21‡	15 *
13	23†	23	23	22‡	22†	22†	17 *	21‡	21‡	15‡*
1-13	23†	23	23	23	22†	22†	18‡*	21‡	21‡	17
No Mangels										
15	23†	23	23	23	22†	22†	21‡	21‡	21‡†	17‡*
16	21††	21†	20††	20††	20††	20††	17‡*†	19‡†	18‡†	16‡*†
18	21 †	21††	20††	20††	20††	20††	20‡*†	20‡†	18 †	14‡*†
19	23†	23	23	23	22†	22†	18 *	21‡	21‡	16‡*
23	23†	23	23	23	23†	23†	21‡	21‡	21‡	17‡*
25	22‡	22‡	21 *	21 *	22†	22†	17‡*	21‡	21‡†	16 *
26	23†	23	23	23	22†	22†	21‡	21‡	21‡	17 *
14-26	23†	23	23	23	22†	22†	18‡*	21‡	21‡	17‡*

\* oxidized, † salty. All other cuts for old or heated flavors.

TABLE 3  
*Frequency of occurrence of oxidized flavor in milk of individual cows of Station Jersey herd when the herd received no mangels for a period of three weeks followed by half the herd receiving mangels and the other half no mangels for the next three weeks*

Age of samples days	Cows 1-13						Cows 14-26					
	Mangel Group						No Mangel Group					
	No. samples tasted	Raw	Past.	No. samples tasted	Past. plus .25 p.p.m. copper		No. samples tasted	Raw	Past.	No. samples tasted	Past. plus .25 p.p.m. copper	
1	35	0	0	23	3		24	0	0	14	1	
3	35	0	2	23	21		24	0	0	14	10	
5	35	0	8	11	11		24	0	2	6	6	
Mangels Fed												
1	30	0	0	30	3		21	0	0	21	2	
3	30	0	5	30	23		21	0	1	21	9	
5	30	3	6	30	27		21	0	0	21	16	

TABLE 4  
Average scores for milk samples from Holstein cows that received no dried beet pulp

Cow No.	Fresh		1 day old		3 days old			5 days old		
	Raw	Past.	Past. plus .25 p.p.m. copper	Raw	Past.	Past. plus .25 p.p.m. copper	Raw	Past.	Past. plus .25 p.p.m. copper	
27	22½	21½	21½	20½	20½	17 *	20	20	17 *	
28	23	23	22½	21½	22	17½*	21½	21½	16½*	
29	23	23	18½*	22	19 *	16½*	20½*	16½*	16½*	
30	23	23	22½	22	22	21½	21½	20½	20½	
31	23	23	22½	22	22	19 *	21½	21½	17½*	
32	23	23	22½	22	22	17½*	21½	21½	17 *	
33	23	33	21½*	22	22	17 *	21½	21½	16 *	
34	23	22½	21½*	21½	21½	17 *	18½	21½	16 *	

\* oxidized. All other cuts for old or heated flavors.

TABLE 5  
Average scores for milk samples from Holstein cows when half of these cows received dried beet pulp

Cow No.	Fresh		1 day old		3 days old			5 days old		
	Raw	Past.	Past. plus .25 p.p.m. copper		Raw	Past.	Past. plus .25 p.p.m. copper	Raw	Past.	Past. plus .25 p.p.m. copper
No Dried Beet Pulp										
27	22½	22½	22½	21 *	22½	22½	16 *	21½	22	16 *
28	22½	22	22	20½ *	22½	22	18½ *	21½	21½	16½ *
29	23	22½	22½	21 *	22½	20½ *	18 *	18½ *	18½ *	17 *
30	23	22½	22½	22½	22½	22	20 *	20	20½ *	17½ *
Dried Beet Pulp										
31	23	22½	22½	21 *	22½	21½	17 *	21½	20½ *	16½ *
32	23	22½	22½	21½ *	22½	22½	20½ *	21½	22	17 *
33	23	22½	22½	19½ *	20 *	19½	18 *	21 *	19 *	17½ *
34	23	22½	22½	20 *	21½	22½	21	21½	19½ *	16½ *

\* oxidized. All other cuts for old or heated flavors.

a little more susceptible to the development of the oxidized flavor than the non-mangel group if it were not for the fact that the relationship of the two groups was the same prior to feeding mangels.

These experiments indicate that the relative freedom from the development of oxidized flavors in the station Jersey herd cannot be attributed to the feeding of mangels.

#### THE EFFECT OF FEEDING DRIED BEET PULP

The experimental procedure used in the preceding experiments with mangels was followed for the eight Holstein cows of the herd. Quart samples of milk were taken from each cow and processed and handled the same as in the preceding experiment and scored for flavor. A set of samples was taken on November 15 and 17 before any dried beet pulp was fed. Dried beet pulp was fed at the rate of three pounds per cow per feed three times a day, two to two and a half hours before milking to cows number 31 to 34, inclusive, commencing November 20 and was continued throughout the test. Samples were taken November 29 and December 1 while dried beet pulp was being fed.

The average scores of the first two sets of samples are given in Table 4. The milk from the individual cows was uniform in flavor with the exception of the milk from cow number 29 which was more susceptible to the development of oxidized flavor and the milk of cow number 27 which carried a little off-flavor.

As can be seen from Table 5 oxidized flavors developed a little more frequently in the milk from both groups than they did in the earlier tests. However, the flavor of the milk from the four Holsteins receiving dried beet pulp was in no way different from the flavor of the milk from the four not receiving beet pulp.

#### CONCLUSIONS

From the results of these tests it is concluded that the feeding of mangels or beet pulp in no way prevented or increased the susceptibility of milk to the development of oxidized flavor.

# MODIFICATION OF THE BLOOM GELOMETER FOR USE IN THE DETERMINATION OF THE CURD TENSION OF MILK

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After a few simple changes have been made, the Bloom gelometer may be used for the estimation of the curd tension of milk, particularly of those milks which yield a very soft curd on coagulation. The gelometer so modified not only reduces the possibility of a personal factor, but also enables the experimenter to conduct within a relatively short time a large number of tests using the same knife.

## DESCRIPTION OF APPARATUS

The Bloom gelometer for the determination of jelly strength was adopted by the Edible Gelatin Manufacturers' Research Society of America in 1923. A detailed description and diagrams of the apparatus were published early in 1924 (4).

A photograph of the modified apparatus together with two of the regular test bottles (4), scales, and the waxed paper cup, used as a receiver for the lead shot, is shown in Figure 1. The receiver weighed only 1.2 grams, since

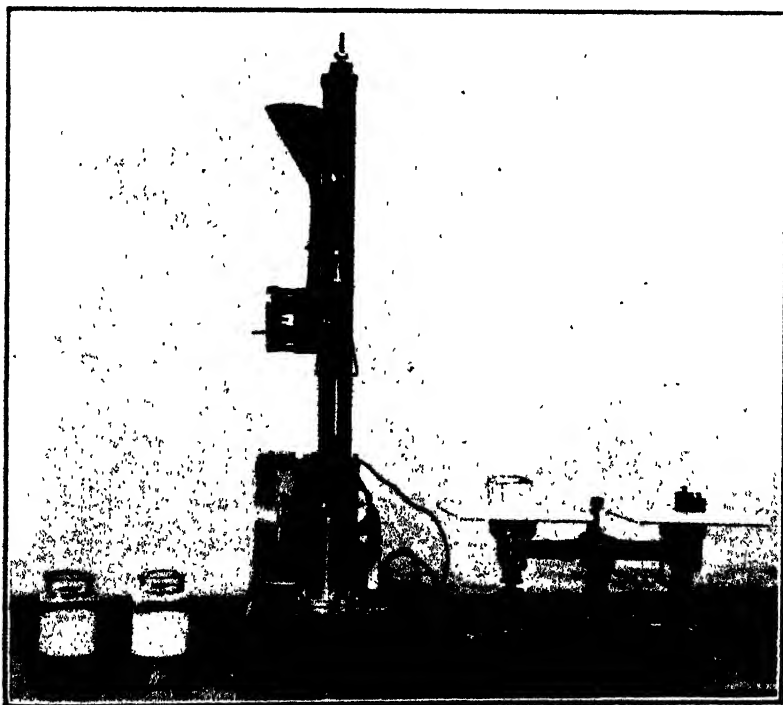


FIG. 1. Modified Bloom Gelometer.

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the weight of a heavy cup might in itself exceed that required to drive the knife through the curd. The rates at which the lead shot were delivered into the receiver at different positions of the dog are recorded in Table 1. The plunger was replaced by a star-shaped knife of the same dimensions as that used with the Hill apparatus (2), so that comparable results might be obtained. The center core of the knife was ground to a needle point extending 10 mm. below the cutting edge. The curd knife, 38 mm. in diameter, was screwed into position with the center needle point and the cutting edges facing downwards. The platform of the gelometer was put on a swivel base so that the test bottle could be placed in position without danger of disturbing the curd. The 4 mm. depression was increased to 17 mm.

#### EXPERIMENTAL PROCEDURE

The milk was coagulated by the method of Hill (2). Before measuring the curd tension, the disk of the gelometer was adjusted so that it just touched the upper contact point. The test bottle containing the milk curd was lifted carefully from the water bath and placed on a towel to remove surface moisture. The platform of the gelometer was swung out toward the experimenter and the test bottle was brought into position so that the curd knife was suspended within the bottle but did not touch the curd. The platform was then swung back and was slowly raised until the test bottle rested on it and the needle point of the curd knife just touched the surface in the center of the curd. The point of contact was taken as that point at which the tip of the needle point just met the tip reflected in the surface of the curd. The current was now turned on and, after ascertaining once more that the disk was just touching the upper contact, the paper cup was placed on the pan and lead shot was allowed to flow into the cup until the tip of the knife had been driven through the curd for a distance of 17 mm., when the flow of shot stopped automatically. The weight in grams of the cup together with the shot was taken as the observed curd tension. To this value was applied a correction of 11.7 grams, the weight required to drive the knife a distance of 17 mm. in the absence of a curd.

In the earliest experiments we attempted to use a procedure similar to that used in the determination of jelly strength. With the disk touching the lower contact, the needle point of the knife was adjusted as described above. The platform was then raised until the disk just touched the upper contact. But with milks of low curd tension, the curd was too soft to support the weight of the knife, so that cutting through the curd had started before the disk reached the upper contact. The curd tension determination was, therefore, begun with the disk at the upper contact.

The possibility existed that the rate of flow of the shot might affect the curd tension reading. A slight difference in the time required to close the spout would introduce a correspondingly greater error with the dog in the

$D_3$  position than in the  $D_1$  position. Moreover, the rate at which the knife was driven through the curd might have some influence on the reading. The curd tensions of two different milks were determined with the dog in each of the three positions. The reconstituted milk was prepared by dissolving 12.8 grams of powdered whole milk (Borden's "Parlac" Brand) in 100 cc. of water. The raw whole milk was a sample of Guernsey milk. The curd tensions were also measured with the Hill apparatus (2). The results are given in Table 1.

TABLE 1

*The effect of the rate of flow of lead shot from the gelometer on the curd tension reading*

Position of dog*	Amount of lead shot delivered in 5 secs.	Curd tension							Curd tension
		Bloom gelometer							Hill apparatus
		Experiment number					Average	Maximum deviation	Average
		(1)	(2)	(3)	(4)	(5)			
	grams			grams			grams	grams	grams
Reconstituted Whole Milk									
$D_1$ ..	57	13	12	15	14	13	13	3	12
$D_2$ ..	135	15	15	14	14	15	15	1	
$D_3$ ..	245	12	17	16	15	14	15	5	
Raw Whole Milk									
$D_1$ ..	57	43	42	45	43	41	43	4	58
$D_2$ ....	135	50	55	46	47		50	9	
$D_3$ ..	245	55	56	58	60	54	57	6	

\* The notations used here:  $D_1$ ,  $D_2$ ,  $D_3$  are the same as those used in the description of the apparatus (4).  $D_1$  designates the lowest dog,  $D_2$ , the dog directly above it, etc.

The rate of flow of shot apparently made little difference under these experimental conditions in the case of milk of very low curd tension. But with milk of higher curd tension there seemed to be a small but consistent increase in the reading as the knife was driven with increasing speed through the curd. The highest value agreed well with that obtained with the Hill apparatus. In our work on curd tension, measurements were made with the dog in the  $D_3$  position.

The results of a series of measurements with the two types of apparatus are recorded in Table 2. The reconstituted whole milk was prepared as described above. The pasteurized whole milk was purchased from a local store. The raw whole milk was obtained from the afternoon milking of Guernsey

cows and was kept at a low temperature until used the following morning. Each value is the average of from two to five concordant results. In nearly every case the agreement between the results obtained by the two methods and recorded in the third and fourth columns of Table 2 is excellent.

TABLE 2

*A comparison of the values obtained for the curd tension of milk with the Hill apparatus and with the modified Bloom gelometer using two kinds of coagulant*

Date	Type of milk	Coagulant					
		Pepsin-calcium chloride			Pepsin-hydrochloric acid		
		Hill apparatus	Modified Bloom gelometer	Difference	Hill apparatus	Modified Bloom gelometer	Difference
		<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>
11/17/37	Reconstituted whole milk						
	Sample 1	12	10	+ 2	8	9	- 1
	Sample 2	11	15	- 4	10	11	- 1
11/18/37	Pasteurized whole milk	28	31	- 3	26	16	+ 10
11/19/37	Pasteurized whole milk	33	32	+ 1	28	16	+ 12
11/21/37	Raw whole milk						
	Cow No. 2	53	54	- 1	55	18	+ 37
	Cow No. 6	49	46	+ 3	48	20	+ 28
	Cow No. 8	45	42	+ 3	43	20	+ 23
	Cow No. 10	45	36	+ 9	42	12	+ 30
	Cow No. 12	42	42	0	41	16	+ 25
	Cow No. 16	37	42	- 5	40	14	+ 26
	Cow No. 20	47	40	+ 7	40	17	+ 23
11/22/37	Pasteurized whole milk	35	33	+ 2	25	18	+ 7
11/24/37	Pasteurized whole milk	34	36	- 2			
11/26/37	Raw whole milk						
	Cow No. 1	39	42	- 3			
	Cow No. 2	41	44	- 3			
	Cow No. 3	39	39	0			
	Cow No. 4	45	38	+ 7			
	Cow No. 5	67	61	+ 6			
	Cow No. 6	72	55	+ 17			
	Cow No. 7	50	52	- 2			
12/3/37	Raw whole milk						
	Cow No. 1	15	18	- 3			
	Cow No. 2	40	39	+ 1			
	Cow No. 3	58	48	+ 10			
	Cow No. 4	60	68	- 8			
	Cow No. 5	45	49	- 4			
	Cow No. 6	71	70	+ 1			
	Cow No. 7	65	50	+ 15			
Average difference ...				+ 2			+ 18

COMPARISON OF PEPSIN-HYDROCHLORIC ACID SOLUTIONS WITH THE PEPSIN  
CALCIUM CHLORIDE SOLUTION AS COAGULANTS IN THE DETERMINATION  
OF THE CURD TENSION OF MILK

Miller (3) recommended the use of a solution of 0.45 per cent pepsin in 0.4 per cent hydrochloric acid for the coagulation of milk in the determination of curd tension. This coagulant resembles the gastric juice more closely than the Hill coagulant.

In the present investigation milk was coagulated with a solution which

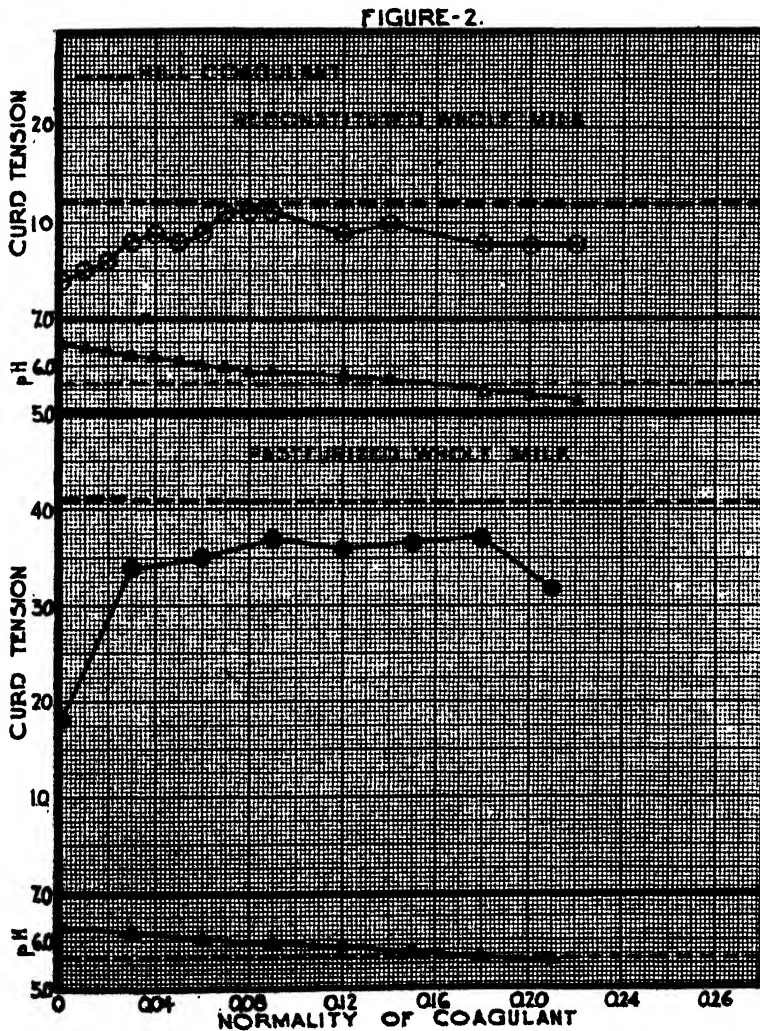


FIG. 2. Effect of the Addition of Pepsin and Varying Concentrations of Hydrochloric Acid on the Curd Tension and the pH of Milk.

contained a constant amount of pepsin and a known but varying amount of hydrochloric acid. The curd hardness was compared with that obtained with the regular Hill coagulant. One cc. of 4.5 per cent pepsin solution and measured quantities of hydrochloric acid of known concentration were pipetted into the test bottles and the volume was diluted with water to 10 cc. During the addition of the 100 cc. of milk the contents of the bottle were given a rotatory motion to ensure complete mixing. After the measurement of curd tension by the Hill apparatus, the curd was thoroughly broken up and the pH of the mixture was estimated with the glass electrode.

The curves of the curd tension and of the pH of two different milks for varying concentrations of hydrochloric acid are given in Figure 2. The addition of pepsin alone to the milk produced a very soft curd. When hydrochloric acid was also used together with the pepsin, the curd tension increased quite rapidly at first and then remained nearly constant over a fairly wide range of pH. The curd tensions of the reconstituted and of the pasteurized milks, which were observed after the addition of the pepsin hydrochloric acid coagulants, were, in general, lower than those obtained with the Hill coagulant. The pH of the milk clot obtained after the addition of the Hill coagulant was lower than that found with the pepsin-hydrochloric acid coagulants, until the concentration of the hydrochloric acid was greater than 0.16 N in the case of the reconstituted milk and about 0.18 N for the pasteurized milks. This agreed well with the observation of Burgwald and Armstrong (1) that the Hill coagulant gave mixtures which were more acid than those formed on the addition of pepsin-hydrochloric acid coagulants, except when the concentration of hydrochloric acid was greater than N/6.67.

The hardness of the curd obtained with the pepsin-calcium chloride coagulant was compared with that produced with a solution containing the same concentration of pepsin in 0.09 N hydrochloric acid. The results are given in the sixth and seventh columns of Table 2. The acid-pepsin coagulant produced a casein curd with a marked tendency toward retraction. With the Hill apparatus the knife tended to lift part of the curd rather than to cut cleanly through it. Greater difficulty was encountered in attempting to measure the hardness of this type of curd with the Bloom gelometer. As the knife passed downward the curd tended to recede, so that for a part of the distance the knife passed through whey rather than curd. As a result the values obtained were much too low. If this type of a curd were to be studied with the Bloom gelometer, it would be necessary to set the knife on the curd itself rather than on the surface of the mixture.

Miller (3) reported that the pepsin-hydrochloric acid coagulant which he used produced a softer curd with boiled milk and a harder curd with raw milk than did the Hill coagulant. However, in our experiments, when the curd tensions of raw milks were measured with the Hill apparatus, the

pepsin-hydrochloric acid coagulant did not give uniformly higher readings than the Hill coagulant.

We wish to thank Dr. Thomas B. Downey for suggesting the use of the gelometer for the estimation of curd tension and for much valuable advice. We are also indebted to Mr. Norman Armstrong for technical assistance.

#### SUMMARY

The Bloom gelometer has been adapted for use in the estimation of the curd tension of milk. It is especially useful in the measurement of very low curd tensions.

When the regular pepsin-calcium chloride coagulant of Hill is used, the modified gelometer gives results which agree well with those of the Hill apparatus.

If a coagulant consisting of 0.45 per cent of pepsin in 0.09 N hydrochloric acid is used, however, the gelometer under the experimental conditions outlined in this paper gives erroneous results, due, in part at least, to greater retraction of the curd produced by this coagulant.

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## SOFT CURD MILK PRODUCED WITH PANCREATIC CONCENTRATE

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The value of soft curd milk in human nutrition has been discussed at length and there have been many and varied attempts and systems of softening the curd of cow's milk. It is generally known that a larger proportion of cow's milk is digested in the stomach of calves in comparison to the amount of human milk digested in the stomach of infants, and it is believed that in order to keep this phenomenon constant these milks are so constituted that this will be possible. The softening of the curd of cow's milk for human consumption is an attempt to make it more nearly like that of human milk with respect to its digestion in infants and adults.

Scales (1) and Carpenter (16) describe three kinds of casein normally present in milk and offer the theory that the change in ratio of these three types of casein may have something to do with the hardness or softness of milk curds. That the digestion of milk is facilitated with the use of soft curd milk is demonstrated by Doan and Welch (2, 3), Espe and Dye (4), and Hill (5). Hill has described the following groups of infants that benefit from the use of soft curd milk: Newly born bottle fed babies, persistent vomiters of whey and leathery curds, colicky babies, the ne'er-do-well group that does not benefit from boiled or sweetened milk, and celiacs and chronic indigestion group and the group that suffers from infantile eczema. Espe and Dye studied the character and activity of the curd in Pavloff pouches of dogs and were able to show that soft curd milk digested more readily than hard curd milk and that the casein content was the factor which affected the nature of the curd more than any of the other milk constituents. Other investigators who discovered that the percentage of casein was very closely related to the type of curd formed in the digestive processes were Scales (1) and The Council on Foods (6), and these investigators generally agree that natural soft curd milk is likewise a low casein milk.

Berry (7) proved that boiling actually lowered the curd tension of milk and also discovered that colostrum was a very hard curd milk. He found that viscolization pressures of 3000 to 5000 pounds were necessary to render hard curd milk soft and this evidence was supported by Theophilus, Hansen and Spencer (8) who decreased the curd tension of milk by homogenization in a single and two-stage homogenizer. Otting and Quilligan (9) described a method of softening the curd by using zeolite sand, during which part of the calcium is removed from the milk thus inhibiting the activity of coagulating enzymes. The Council on Foods (6) also brings out that milk curd may

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be softened by dilution with water, the addition of acids and alkalies and the addition of various cereal extracts.

Lahrmann (10) in making a substitute for mother's milk used digestive ferments. He very nearly completely digested the casein and albumen and added water, sugar, cream, potassium carbonate and phosphoric acid. Von Dungern (11) produced a soft curd milk by coagulating the milk with rennet and then dispersed the curd particles by a mechanical means. Backaus (12) showed that by the addition of alkalies and trypsin to milk in combination with cream and lactose he was able to produce a soft curd milk. Thew (13) produced a soft curd milk by peptonization accompanied by the addition of sodium bicarbonate and condensation. Turney (14) also produced a soft curd milk by the use of a curdling enzyme and concentration of the milk by draining off the whey.

#### PLAN OF EXPERIMENT

The processes for producing soft curd milk described above have certain disadvantages in that they require special equipment and are in general lengthy procedures. The severe enzyme treatment as recommended by Lahrmann results in the formation of bitter proteoses, peptones and amino acids. Our problem was one of producing a soft curd milk, at a reasonable expense and by a method which would fit into the ordinary dairy plant, without taking away any of the natural nutritive value or minerals of the milk.

The use of digestive enzymes presented itself favorably, in view of the fact that small amounts could be used and there was a possibility that a method could be found for using them in milk plant operations. Preliminary experiments showed that the use of specially prepared (high tryptic, low diastatic) pancreatic enzymes in dilutions of 1-6500 reduced the curd tension considerably but the reaction between the milk protein and the enzyme continued upon the storing of the milk and produced a bitter flavor when the enzyme was added after pasteurization. This brought out the necessity for determining how the enzyme should be inactivated and what the fate of the enzyme plus milk would be under pasteurization conditions.

It was determined that milk could be pasteurized in the presence of pancreatin if the dilution of enzyme with milk was high enough. The inactivation of pancreatin was determined by making dilutions of enzyme with milk and heating the milk to temperatures varying from 62.5 to 73.0° C. The curd softening process was studied from the standpoint of the dilution factor, temperature and time of incubation, type of enzyme used, the storage properties of the softened milk, the effect on the calcium-phosphorus, calcium-magnesium, calcium-protein ratios, formol titration, dialyzability of inorganic material, and the in vivo reaction of the softened milk in calves' stomachs.

## METHOD

The method which was used in the curd softening process was devised so that it could be applied with either the flash or the holder method of pasteurization. A typical batch would be prepared by weighing out enough of the pancreatic concentrate to make a dilution of 1-10,000 when incorporated into the milk. This finely ground powder is diluted with enough water so that there are no large particles remaining on the surface and this in turn is poured into the milk to be softened at a temperature of 43° C. The milk is then allowed to incubate for 15 minutes, which is followed by the regular pasteurization process. The temperature used in the flash method was 73.0° C. and the temperature used in the holder was 62.5° C.

The curd tension of the milk was measured by the method of Hill (15) and although this method was found to have various sources of error, each curd tension determination consisted of running four samples and by taking the average of these consistently accurate results were obtained. The data compiled in Table 2 were founded upon the methods recommended by the Association of Official Agricultural Chemists, with the exception of the Walker method which was used in the formol titration.

## RESULTS

The results of the inactivation experiments showed that if the milk plus enzyme were held at a temperature of 73° C. for 15 seconds 90 to 95 per cent of the enzyme was inactivated, and if the milk plus enzyme were held at 62.5° C. for  $\frac{1}{2}$  hour similar results were obtained. The pasteurization of milk in the presence of pancreatin was possible with dilutions as low as 1-5000.

The data incorporated in Table 1 show that milk curd tension could be lowered consistently to an average of 21.7 grams of tension when the flash and holder method of pasteurization are considered jointly. The decrease in curd tension was accomplished in a variety of ways and with respect to the dilutions used favorable results were obtained with values ranging from 1-6500 to 1-50,000. When the holder method of pasteurization is employed a higher dilution of enzyme can be used. The flash type of pasteurization was used best in conjunction with a 15-minute incubation period at a temperature of 42° C. The time and temperature of incubation required for pasteurization by the holder method was found to have little significance on the amount of reduction in curd tension and this was varied from 24 hours at 7° C. to 15 minutes at 43° C. The average curd tension of the raw milk was approximately 50 grams. Upon storing milk softened by this method it was found that the curd tension decreased an average of 5.6 grams in 72 hours.

The curd tension of milk could be reduced only 5 or 6 grams by the use of diastase when the dilution of diastase was 1-250 and 1-500. These ex-

TABLE 1  
*Data of soft curd milk produced with variations in dilutions, incubation, and pasteurization*

No. of samples	Enzyme used	Dilution in milk	Temp. of incubation °C.	Time of incubation	Temp. of inactivation °C.	Average tension of control	Average tension of softened milk	Average tension after 72 hours
24	Pancreatin	1-6500	36°	15 min.	74° Flash	60 grams	25 grams	23 grams
15	"	1-6500	31°	15 "	76° Flash	50 "	22 "	"
15	"	1-10,000	43°	15 "	76° 6 min.	55 "	22 "	"
20	"	1-10,000	42°	25 "	76° Flash	50 "	28 "	15 "
15	"	1-20,000	42°	30 "	73° Flash	40 "	28 "	18 "
15	"	1-30,000	42°	30 "	73° Flash	36 "	22 "	26 "
30	"	1-29,000	7°	24 hrs.	62.5° 30 min.	40 "	26 "	20 "
30	"	1-30,000	7°	24 "	62.5° 30 min.	42 "	28 "	24 "
20	"	1-20,000	none	none	62.5° 30 min.	52 "	30 "	21 "
25	"	1-20,000	15°	6 hrs.	62.5° 30 min.	40 "	30 "	24 "
16	"	1-15,000	none	none	62.5° 30 min.	38 "	26 "	24 "
28	"	1-40,000	7°	24 hrs.	62.5° 30 min.	42 "	30 "	22 "
28	"	1-50,000	7°	24 "	62.5° 30 min.	46 "	30 "	24 "

periments on diastase were carried out with the use of a 15-minute incubation period and the flash method of pasteurization.

Other enzymes of vegetable and animal origin such as rennin, pepsin, papain, etc., were used with the same methods of pasteurization and results which were comparable but usually inferior were obtained.

The supernatant fluid obtained from mixing powdered pancreatic concentrate and water was found to have a slight curd softening effect but the decrease in curd tension was far less than that obtained when the dried particles of the pancreatic concentrate were included in the softening material. By allowing the powdered concentrate to soak in water for from one to two hours there was a slight increase in the amount of softening activity but this was found to be of only minor significance.

This method of producing soft curd milk has been tried out on a commercial scale using 100 gallon batches in a large dairy plant and has been found to be practical in all respects. The fat and acidity contents of the milks used were  $3.6\% \pm 0.3\%$  and  $0.16\% \pm 0.02\%$ , respectively. Ammonium carbonate was dissolved in the water into which the pancreatin was mixed and it was found to have an inhibitory action on the curd softening enzymes. No other alkaline materials were used in an attempt to adjust the pH of the water.

Sixteen calves were used in a feeding test, each calf receiving four 1 gallon lots of milk during four feedings. The milk used in the third feeding was colored red and the milk used in the fourth feeding was colored blue so that when the calves were killed seven hours after the last feeding the amount of curd which remained in the stomach from each feeding could be determined. The calves which were fed normal pasteurized milk retained 23 per cent more curd in their stomachs than did the calves which were fed soft curd milk and the tension of the normal curds, as measured by the Hill Curdometer, was twice as great as the enzyme curds. Calves were used in this test because they were thought to be a natural testing medium for cow's milk, and because of the possibility of examining the stomach contents after slaughter.

The data incorporated in Table 2 show the relative value of  $P_2O_5$ , protein, calcium, magnesium and formol titration of the control milk in comparison with the enzymatically softened milk and zeolite softened milk. These milks were also dialyzed and the dialysate analyzed for  $P_2O_5$ , calcium and magnesium. The whey from the normal and enzyme treated samples was also analyzed for  $P_2O_5$ , calcium and magnesium. The  $P_2O_5$  analyses show only small variations. This was found to be true also with the analysis for protein and the formol titration. The results of the magnesium determinations show that probably the only significant feature demonstrated is that the zeolite dialysate samples contained no magnesium. There was such a slight variation in the calcium values of the wheys and dialysates that here

TABLE 2

*Calcium, magnesium, phosphorus, protein and formol titration values of normal, enzyme treated, and zeolite treated milk\**

Sample	P <sub>2</sub> O <sub>5</sub> %	Ca %	Mg %	Protein (Kjeldahl N × 6.25) %	Formol titration (Walker) (10 cc. sample) cc. N/10 NaOH
Normal Control Milk A ... ..	.126	.190	.009	3.13	1.1
Normal Control Milk B .....	.167	.188	.008	3.07	1.2
Enzyme Softened Milk A .....	.150	.194	.007	3.02	1.3
Enzyme Softened Milk B .. .	.149	.196	.007	3.00	1.5
Zeolite Milk I .....	.131	.157	.009	2.97	1.2
Zeolite Milk II .....	.133	.159	.009	2.72	1.3
Dialysate of Control Milk A ..	.036	.015	.006		
Dialysate of Control Milk B ..	.032	.021	.005		
Dialysate of Enzyme Softened Milk A .....	.038	.026	.004		
Dialysate of Enzyme Softened Milk B .....	.031	.023	.003		
Dialysate of Zeolite Softened Milk I .....	.033	.032	.000		
Dialysate of Zeolite Softened Milk II .....	.038	.043	.000		
Whey of Control Milk A (rennet coagulation) .....	.088	.070	.007		
Whey of Control Milk B (rennet coagulation) .....	.087	.065	.005		
Whey of Enzyme Softened Milk A .....	.089	.075	.009		
Whey of Enzyme Softened Milk B .....	.090	0.85	.005		

\* All samples in duplicate. All "A" and "B" samples represent split batches of the same milk.

too no outstanding significance is demonstrated. The calcium values of the zeolite softened milk samples were found to contain 18 per cent less calcium than the control samples and the enzyme treated samples.

Clinical investigations on a milk prepared by this method are being carried out.

#### CONCLUSIONS

The curd tension of ordinary cow's milk may be softened by pancreatic concentrate to within a range of 20 to 30 grams and the curd tension of samples of milk which have been softened enzymatically decrease in their curd tension value upon storage.

Either the flash or holder type of pasteurization may be employed in the method described above.

Calves fed milk which has been softened with pancreatic concentrate retain the curd in their stomachs for a shorter period of time and the curd formed therein is much softer than the curd formed when normal pasteurized milk is fed.

The  $P_2O_5$ , calcium, magnesium, protein and formol titration values are changed so slightly from that of pasteurized milk that they are of little significance in studying the nature of enzymatically softened milk.

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# THE CHEMICAL COMPOSITION AND PROPERTIES OF NORMAL AND RANCID JERSEY MILK

## III. TITRATABLE ACIDITY, HYDROGEN-ION CONCENTRATION AND LIPASE CONTENT

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A study has been made of the chemical composition of the milk of a Jersey herd in relation to the off-flavor, rancidity. In the course of the study normal values were established for several constituents of milk, both for individuals and for the herd throughout the entire lactation period. The composition of rancid milk has been compared with that of normal milk of the same individual and of the herd. The chloride, lactose, fat, total solids and protein content of the milk has been reported in previous papers (3) (4). The present paper discusses the titratable acidity, hydrogen-ion concentration and lipase content of normal and rancid milk.

Published data indicate a wide range in the titratable acidity and pH of normal milk. This variation is attributed to such factors as breed, environment, period of lactation, and individuality. In the present study the number of variable factors was reduced by employing a single breed of cows, by maintaining them under the same environmental conditions and by comparing the composition of rancid milk with that of normal milk produced in the same period of lactation.

### EXPERIMENTAL

Management of animals and methods of obtaining milk samples have been described in a previous paper (3). Over a period of eight months, determinations were made of the titratable acidity and pH values of samples taken weekly from each of 18 members of the Jersey herd. Lipase determinations were made over a period of 20 months.

Titratable acidity was determined by titrating 10 cc. of the fresh sample with 0.1 N NaOH, using phenolphthalein as an indicator. The degree of acidity of the sample is expressed as the percentage of lactic acid. pH was determined by use of the quinhydrone electrode. Methods used in estimation of the lipolytic activity of milk are discussed later.

### PRESENTATION OF DATA

#### *Titratable Acidity and Hydrogen-Ion Concentration of Normal Jersey Milk*

The mean titratable acidity and pH values of all milk samples obtained from each of 12 animals are shown in Table 1. The mean acidities ranged

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from 0.158 per cent to 0.112 per cent; individual samples showed minimal and maximal values of 0.122 per cent and 0.410 per cent, respectively. The mean pH values ranged from 6.60 to 6.43, with minimal and maximal values of 6.56 and 6.10 for individual samples.

Monthly changes in the mean titratable acidity and pH values of the normal milk of the herd are shown in graphs 1 and 2, Figure 1. The general

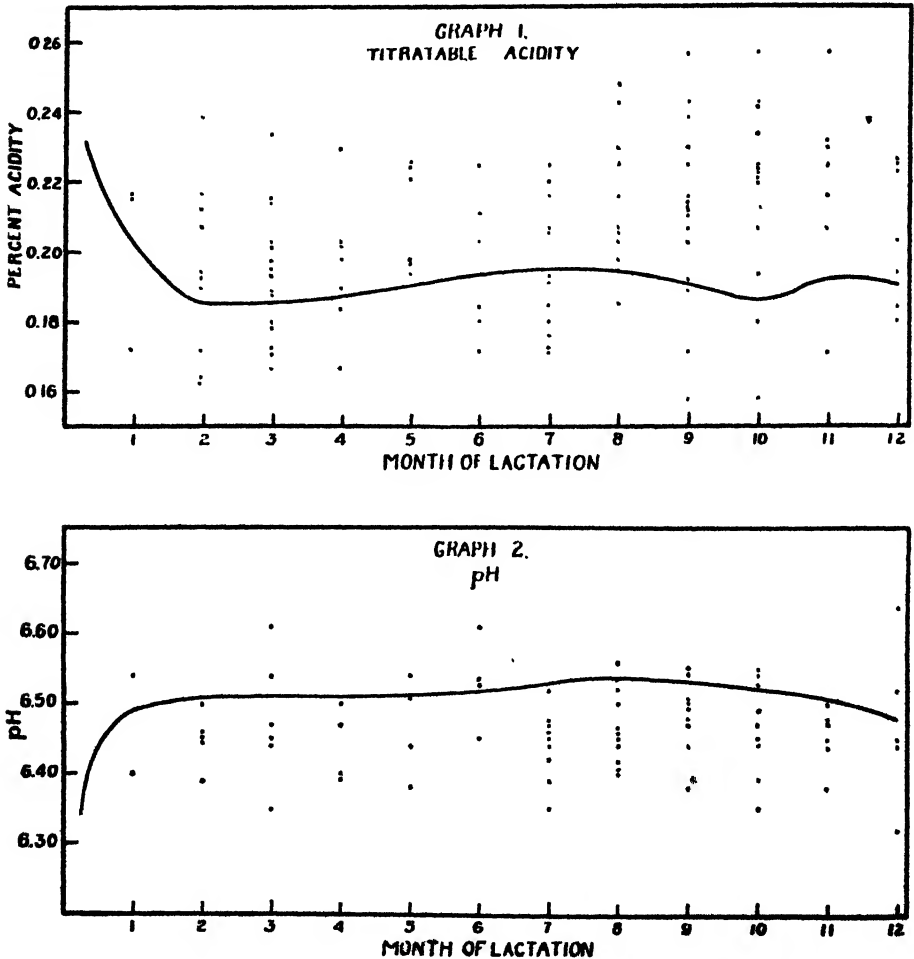


FIG. 1. Titratable acidity and pH of normal and rancid Jersey milk.

#### Graph 1

- Mean titratable acidity of all normal samples for each month of the lactation period.
- Titratable acidity of rancid samples.

#### Graph 2

- Mean pH of all normal samples for each month of the lactation period.
- pH of rancid samples.

trends of titratable acidity and hydrogen-ion concentration throughout lactation were similar, both showing a rapid decrease at the beginning of lactation, with little change thereafter. During the first six weeks the mean titratable acidity dropped from 0.234 per cent to 0.185 per cent, and the pH increased from 6.34 to 6.53, after which they remained nearly constant until the end of lactation.

The above data are summarized in Table 2, which shows the mean titratable acidity, hydrogen-ion concentration and pH of all normal samples for each of twelve four-week periods. The coefficients of variation indicate a marked variation in milk from different animals in the same period of lactation. The mean titratable acidity of 357 normal samples was  $0.189 \pm 0.004$  per cent with a standard deviation of  $\pm 0.027$ , and a coefficient of variation of 20.6; the mean hydrogen-ion concentration was  $3.05 \pm 0.04 \times 10^{-7}$  (pH 6.52) with a standard deviation of  $\pm 0.63$  and a coefficient of variation of 14.2.

Caulfield and Riddell (1) have reported values for the titratable acidity of the milk of six Jersey cows as determined by samples collected at monthly intervals throughout a complete lactation. Their values are in close agreement with those reported here. They found an average titratable acidity of 0.179 per cent, individual variation in average acidities of from 0.098 per cent to 0.295 per cent, and a range of from 0.194 per cent in the first month to 0.145 per cent in the tenth month of lactation. They also observed a marked drop in titratable acidity between the first and second months of lactation, after which the acidity remained nearly constant through the seventh month. During the last month of lactation they found a marked decline in the average acidity of milk. Such a decline was not observed in the present study; instead, a slight increase in titratable acidity manifested itself in the milk of animals whose lactation period was unduly prolonged.

The fairly wide deviation in the pH of milk is well recognized. Sommer (5) in a recent study observed a range of from 6.77 to 6.22 in the pH values of 386 milk samples obtained from 43 cows (breed not specified). 140 of the samples had pH values of from 6.36 to 6.45 and 144 from 6.46 to 6.55. These values are in agreement with those observed in the present study.

#### *Titratable Acidity and Hydrogen-Ion Concentration of Rancid Jersey Milk*

As may be seen in Table 1, five animals produced no rancid milk during the period in which titratable acidity and pH were determined. The mean titratable acidity of their milk varied from 0.158 per cent to 0.203 per cent, with an average value of 0.185 per cent; the pH values ranged from 6.60 to 6.47, with an average of 6.54. Seven animals produced rancid milk comprising 19 to 100 per cent of all samples obtained from them. The mean titratable acidity of their milk ranged from 0.162 per cent to 0.212 per cent and averaged 0.194 per cent; the pH values varied from 6.53 to 6.43, with

an average of 6.48. In considering the milk of the individual animals, only in the case of cows 6 and 11 was the average titratable acidity of rancid samples appreciably higher than that of the normal milk. The pH of the rancid milk of animals 6, 9 and 10 was appreciably lower than that of their normal milk.

To facilitate comparison with normal values for the period of lactation in which they occurred, the titratable acidity and pH values of rancid samples are indicated as separate points on the graphs in Figure 1. From the graphs it is evident that rancid samples tended to have higher titratable acidities and lower pH values than did normal milk of the same period of lactation.

In Table 3 rancid samples are grouped according to their degree of rancidity. Samples described as "very slight" or "doubtfully rancid" had practically the same mean titratable acidity and hydrogen-ion concentration as did normal milk. Those criticized as "very rancid," "rancid," and "slightly rancid" had a mean acidity of 0.212 per cent as compared to 0.189 per cent, the mean acidity of all normal samples; their mean pH was 6.41 as compared with 6.51, the mean value for normal milk. Both the mean titratable acidity and the hydrogen-ion concentration of all rancid samples were significantly higher than the mean values of all normal samples.

#### METHODS OF ANALYSIS

Hileman and Courtney (2) have recently reviewed previous work on the lipase content of milk. In the present study an attempt has been made to secure a method capable of detecting the presence of small amounts of lipase and to provide a standard with which the lipolytic activity of milk might be compared.

Two methods of estimation were employed in the study of the lipase content of milk. The first was an adaptation of the general procedure of Willstätter, Waldschmidt-Leitz and Memmen (6). The lipolytic activity of a known volume of milk was estimated by determining the amounts of free fatty acid liberated by the hydrolysis of olive oil in the presence of an accelerator. Determinations were made in the following manner. 2.5 gms. of olive oil, 2 ml. of an ammonium chloride-ammonium hydroxide buffer, pH 8.9, 1 ml. of a one per cent calcium chloride solution and 10 ml. of milk were shaken vigorously for 3 minutes, and then placed in a water bath at 30° C. At the end of the incubation period the contents of the flask were washed into a 250 ml. Erlenmeyer flask with 112 ml. of 95 per cent alcohol. Twenty ml. of ether and 1 ml. of a saturated solution of potassium oxalate were added and the fatty acids titrated with N/10 alcoholic NaOH, thymolphthalein being used as an indicator. The amount of acid produced was determined by subtracting the initial acidity of the reaction mixture, determined in controls, from the acidity of the reaction mixture following incubation.

To ascertain the minimum amount of lipase detectable by this method,

determinations were made of the degree of hydrolysis produced by decreasing amounts of a commercial preparation of lipase during a one hour's incubation period. In the first series of experiments an aqueous medium was employed. The addition of 240, 160, 100, 80, 60 and 40 mgs. of the lipase preparation produced amounts of acid equivalent to 18.0, 15.0, 10.0, 8.0, 6.5 and 4 ml. of 0.1 N NaOH, respectively; the lipolytic activity of amounts less than 20 mgs. is shown by curve 1, Figure 2.

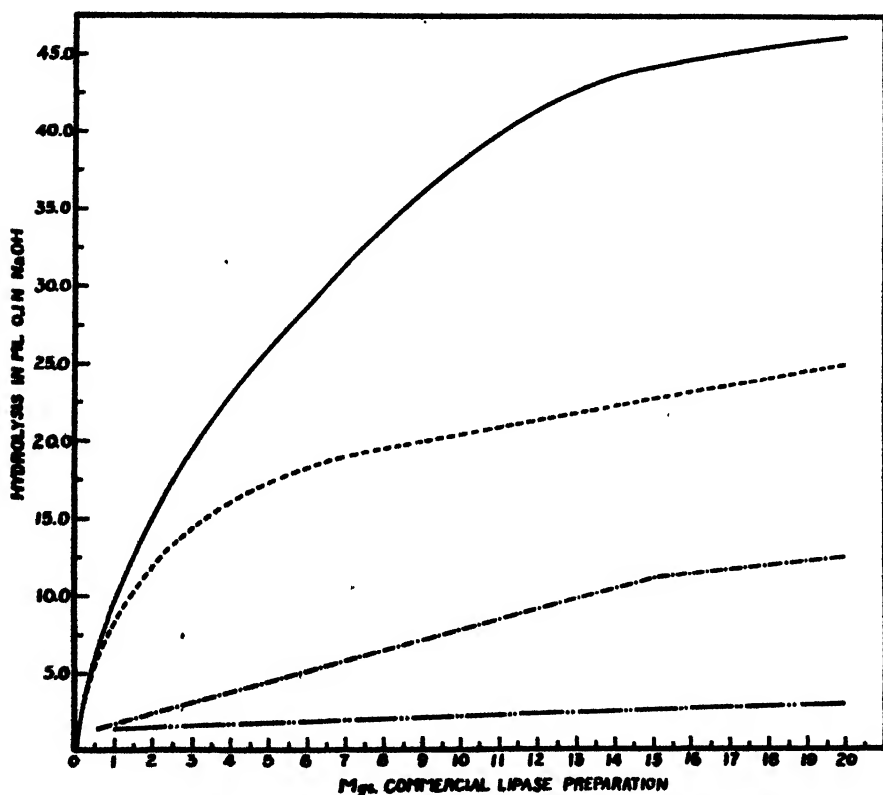


FIG. 2. Hydrolysis of olive oil and tributyrin by commercial lipase preparation in media of milk and water.

- Aqueous medium, olive oil substrate, 1 hour incubation.
- - - Milk medium, olive oil substrate, 1 hour incubation.
- · - Milk medium, olive oil substrate, 24 hours incubation.
- Milk medium, tributyrin substrate, 24 hours incubation.

In the second series of experiments, inactivated milk served as the medium. Inactivation was effected by heating the milk for one hour at 70° C. Curve 2, Figure 2, shows the degree of hydrolysis produced in one hour by amounts of lipase preparation ranging from 20 mgs. to 0.6 mgs.

The emulsion of milk and olive oil proved to be more favorable to the action of lipase than the emulsion of water and olive oil. By increasing the

period of incubation from one to 24 hours, it was possible, using milk as a dilutant, to detect the presence of 0.06 mg. lipase preparation, as may be seen from curve 3, Figure 2. A 24-hour incubation period was therefore adopted.

In a second method later employed for the estimation of lipase, two 50 ml. aliquots of each milk sample were used, one aliquot serving as a control. The control was heated for one hour at 70° C. to inactivate the lipase present. To each of the aliquots were added 50 ml. water, 0.5 ml. tributyrin and 4 drops of formaldehyde. The two reaction mixtures were then shaken for three minutes and incubated for 24 hours at 30° C., after which they were diluted and titrated as described above. The difference between the titration values of the raw and inactivated samples after incubation was taken as a measure of the degree of hydrolysis produced.

To determine the smallest amount of lipase which could be detected by this method, known amounts of lipase preparation were added to previously inactivated milk. Lipase added to the controls was also inactivated. Results obtained by this method are given in Curve 4, Figure 2. This method proved as sensitive as the first method for amounts of the lipase preparation less than 1 mg. and more sensitive for amounts greater than 1 mg.

#### RESULTS

The two methods described above were used in estimating the lipase content of 490 samples of milk, 121 of which were rancid. The results are given in Table 4. In this table samples are grouped according to flavor and without regard to the period of lactation, since there appeared to be no correlation between the period of lactation and the amount of lipase present. Table 4 also includes the amount of commercial lipase preparation which under the same conditions produced a corresponding degree of hydrolysis. These amounts were read from curves 3 and 4, Figure 2, when methods 1 and 2 respectively, were employed.

The results indicate that all samples had a definite lipolytic action which was, however, very small when compared with a commercial lipase preparation. As determined by the first method, 10 ml. of normal milk produced the same degree of hydrolysis as did approximately 0.15 mgs. of the lipase preparation; when the second method was employed 50 ml. of normal milk showed a lipolytic activity equivalent to that of 0.4 mg. of lipase preparation.

By both methods, samples described as "very slightly" or "doubtfully" rancid were found to have a lipolytic activity approximately equal to or somewhat less than that of normal milk; "slightly" rancid samples produced a somewhat greater amount of acid than did normal milk, differences in the average titrations being 0.8 mg. and 0.9 mg. for the two methods. Twenty-seven "very rancid" and "rancid" samples analyzed by the first procedure showed about twice as much lipolytic activity as normal milk. One "very

TABLE 1

*Titratable acidity and pH values of normal and rancid milk of 12 Jersey cows*

Cow number	Milk samples		Mean titratable <sup>1</sup> acidity			Mean pH		
	Normal	Rancid	Normal	Rancid	Total	Normal	Rancid	Total
	number	number						
1	20	0	0.176		0.176	6.55		6.55
2	35	0	0.158		0.158	6.60		6.60
3	29	0	0.203		0.203	6.51		6.51
4	18	0	0.189		0.189	6.56		6.56
5	27	0	0.203		0.203	6.47		6.47
6	21	4	0.198	0.275	0.198	6.52	6.33	6.47
7	24	4	0.203	0.203	0.203	6.51	6.47	6.51
8	15	7	0.176	0.185	0.180	6.49	6.53	6.50
9	22	9	0.212	0.216	0.212	6.46	6.35	6.43
10	9	17	0.162	0.167	0.162	6.57	6.46	6.53
11	14	18	0.194	0.225	0.212	6.47	6.47	6.47
12	0	30		0.203	0.203		6.48	6.48

<sup>1</sup> Expressed as percent lactic acid.

TABLE 2

*Titratable acidity and hydrogen-ion concentration of normal<sup>1</sup> Jersey milk in relation to the period of lactation*

Lactation periods	Milk samples	Titratable acidity <sup>2</sup>			Hydrogen-ion concentration ( $\times 10^{-7}$ )			pH <sup>6</sup>
		Mean	S. D. <sup>3</sup>	C. V. <sup>4</sup>	Mean	S. D. <sup>3</sup>	C. V. <sup>4</sup>	
weeks	number			percent			percent	
1-4	40	$0.203 \pm 0.005^5$	0.035	17.3	$3.68 \pm 0.13^5$	0.84	22.8	6.43
5-8	48	$0.185 \pm 0.004$	0.024	12.9	$3.05 \pm 0.08$	0.54	17.6	6.51
9-12	27	$0.185 \pm 0.005$	0.022	11.7	$3.07 \pm 0.07$	0.49	16.0	6.51
13-16	36	$0.194 \pm 0.005$	0.030	15.0	$2.96 \pm 0.05$	0.35	12.0	6.53
17-20	32	$0.185 \pm 0.005$	0.029	15.7	$3.01 \pm 0.11$	0.74	24.5	6.52
21-24	30	$0.194 \pm 0.005$	0.031	15.6	$3.10 \pm 0.09$	0.57	18.3	6.51
25-28	22	$0.194 \pm 0.005$	0.022	11.1	$2.92 \pm 0.06$	0.42	14.5	6.53
29-32	26	$0.194 \pm 0.006$	0.029	11.1	$2.89 \pm 0.06$	0.41	14.3	6.54
33-36	28	$0.185 \pm 0.005$	0.023	15.0	$2.74 \pm 0.07$	0.45	16.4	6.56
37-40	14	$0.180 \pm 0.006$	0.023	12.7	$3.07 \pm 0.08$	0.55	17.9	6.51
41-44	10	$0.194 \pm 0.005$	0.024	12.8	$3.01 \pm 0.07$	0.45	15.2	6.52
45-48	13	$0.185 \pm 0.006$	0.023	11.7	$3.29 \pm 0.18$	1.19	36.0	6.48

<sup>1</sup> No rancid samples are included.<sup>2</sup> Expressed as percent lactic acid.<sup>3</sup> Standard deviation of mean.<sup>4</sup> Coefficient of variation of mean.<sup>5</sup> Standard error of mean.<sup>6</sup> Corresponding to the mean hydrogen-ion concentration for the period.

rancid" sample analyzed by the second method produced approximately ten times as much acid as did normal milk. This was, however, the only sample to display such a marked degree of activity.

One would conclude, therefore, that all milk contains lipolytically active

TABLE 3

*Titratable acidity and hydrogen-ion concentration of normal and rancid Jersey milk*

Description of samples	Milk samples	Titratable acidity <sup>1</sup>		Hydrogen-ion concentration ( $\times 10^{-7}$ )			pH <sup>4</sup>
		Mean	Standard deviation	Milk samples	Mean	Standard deviation	
	<i>number</i>			<i>number</i>			
Very rancid, rancid and slightly rancid .....	84	$0.212 \pm 0.005^2$	0.035	56	$3.89 \pm 0.17$	1.30	6.41
Very slightly and doubtfully <sup>3</sup> rancid .....	47	$0.194 \pm 0.004$	0.023	21	$3.20 \pm 0.09$	0.48	6.49
All rancid samples	131	$0.203 \pm 0.003$	0.033	77	$3.68 \pm 0.09$	1.17	6.43
Normal .....	357	$0.189 \pm 0.002$	0.027	211	$3.05 \pm 0.04$	0.63	6.51

<sup>1</sup> Expressed as percent lactic acid.

<sup>2</sup> Standard error of mean.

<sup>3</sup> Samples criticized as rancid by less than half the judges.

<sup>4</sup> Corresponding to the mean hydrogen-ion concentration.

TABLE 4

*The lipolytic activity<sup>1</sup> of normal and rancid Jersey milk*

Description of samples	Method 1. (10 ml. milk)			Method 2. (50 ml. milk)		
	Milk samples	Hydrolysis in ml. 0.1 N NaOH	Commercial lipase equivalent <sup>2</sup>	Milk samples	Hydrolysis in ml. 0.1 N NaOH	Commercial lipase equivalent <sup>2</sup>
	<i>number</i>		<i>mg.</i>	<i>number</i>		<i>mg.</i>
Very rancid .....	1	4.2	0.40	1	43.6	14.6
Rancid .....	26	2.8	0.25	3	9.7	1.0
Slightly rancid .....	32	2.3	0.20	25	5.4	0.5
Very slightly rancid	10	1.5	0.15	9	3.9	0.4
Doubtfully <sup>3</sup> rancid ..	13	0.9	0.10	5	3.8	0.4
Normal .....	159	1.5	0.15	210	4.5	0.4

<sup>1</sup> Expressed in terms of ml. 0.1 N NaOH required to neutralize the free fatty acids produced during a 24-hour incubation period.

<sup>2</sup> Amount of commercial lipase preparation producing a degree of hydrolysis equal to that produced by milk samples under the same conditions.

<sup>3</sup> Samples criticized as rancid by less than half the judges.

substance and that milk which has a definitely rancid flavor is somewhat more active lipolytically than is normal milk.

#### SUMMARY AND CONCLUSIONS

Data have been presented showing the titratable acidity, hydrogen-ion concentration and lipase content of the milk of animals of a Jersey herd, all of which received the same ration and were subject to the same environmental conditions. The amounts found in milk criticized as rancid have been compared with those present in normal milk of the same period of lactation.

Rancid milk was found usually to have a higher titratable acidity and hydrogen-ion concentration than normal milk of the same period of lactation. The mean titratable acidity and hydrogen-ion concentration of all rancid samples were significantly higher than the mean values for all normal samples.

All milk was found to contain a small amount of lipolytically active substance. Definitely rancid milk was somewhat more active lipolytically than was normal milk.

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# A COMPARATIVE STUDY OF METHODS OF DETERMINING THE MOISTURE CONTENT OF CHEDDAR CHEESE

## II. THE STEAM OVEN METHOD AT HIGH PRESSURE AND THE OLIVE OIL METHODS\*

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### INTRODUCTION

In a previous study (1) a discussion of the use of an open flame-olive oil method for the determination of moisture in Cheddar cheese was given and its accuracy compared with the Mojonnier method, slightly modified. In addition, a modification of the oil method was suggested in which a small amount of sodium chloride was added to the oil. The addition of the salt prevented the cheese from lumping and sticking during heating, and also prevented the cheese from spattering. The olive oil method gave results approximately 0.3 per cent above those secured by the Mojonnier method, whereas the oil method, modified by the addition of salt, gave results averaging approximately 0.55 per cent higher than the results by the Mojonnier procedure.

The steam pressure oven method for moisture determination was originally developed for butter (2), but has since been more widely adopted for cheese analysis. Sammis (3) (4) gives in detail the steps involved in the moisture determination of cheese by this method, and points out that a 10 gram sample is dried satisfactorily in at least 4 to 5 hours and a 5 gram sample in about one-half of this time when the steam pressure ranged from 50 to 60 pounds. According to this author, excessive heating periods in the steam pressure oven had little effect on new cheese, but old cured cheese continued to lose weight at a noticeable rate. Van Slyke and Price (5) point out that disastrous effects on the samples may occur if the oven is connected directly with a boiler carrying a high steam pressure without having an intervening valve. Sammis (3) in discussing the steam pressure oven method, states that "duplicates commonly agree with each other within 0.5 per cent."

In connection with the study of the olive oil method previously reported (1), samples of Cheddar cheese which were analyzed for moisture by the oil procedure were also dried to constant weight by the use of a steam pressure oven. It was thought desirable to tabulate the data collected and to compare the results obtained by these methods.

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## EXPERIMENTAL

The cheese was prepared and kept for analysis according to directions previously outlined (1). The moisture analysis by use of the steam pressure oven was made on a 2-3 gram sample which was weighed in an aluminum dish approximately 55 mm. in diameter, 22 mm. in height, and provided with a slip-in inverted cover fitting tightly on the inside. The drying was carried out in a Farrington steam pressure oven connected directly to the main line which carried a steam pressure of approximately 85 pounds. Two and one-half hours were usually required to bring the samples to constant weight.

Analysis of the cheese by the olive oil method was carried out (a) by use of a 5 gram sample of finely chopped cheese in 20 cc. of olive oil; and (b) by use of 5 grams of cheese in 20 cc. of olive oil which contained approximately 1 gram of sodium chloride. The latter procedure will be referred to as the modified olive oil method. More detailed information concerning the procedure for the oil methods has been given (1).

*Differences between Duplicates:* Excellent checks between duplicate determinations were obtained by the steam oven method. The average difference between duplicates of 29 trials by this method was  $0.19 \pm .02$  per cent with 18 or approximately 62 per cent of the duplicates varying 0.2 per cent or less. Only one of the 29 trials showed variation between duplicates greater than 0.5 per cent. The variations one might expect by the use of the olive oil method were discussed previously (1) in which it was found that in 28 trials, the average difference between duplicates by the regular oil method was found to be  $0.26 \pm .03$  per cent. Sixty-four per cent of these duplicates varied not greater than 0.2 per cent. In the case of the modified olive oil method, 63 per cent of the duplicates gave variations which fell within this range, with the average difference of 20 trials being  $0.20 \pm .03$  per cent.

*Comparison of Results:* The results of the analysis of Cheddar cheese by the steam pressure oven method, by the olive oil method, and by the modified olive oil method are given in Table 1.

The results show the steam pressure oven method, at the high pressure operated, gave results averaging approximately 0.3 per cent above those secured by the regular olive oil method. The average of the individual differences for the 31 trials was somewhat greater, averaging  $0.49 \pm .04$  per cent. That the oven method tends to give higher results is shown by the fact that 28 of the 31 trials gave higher values by the steam oven method than by the regular olive oil procedure.

The modified olive oil method gave results which for 17 trials averaged within 0.1 per cent of the steam oven method. The averages of the differences between the results for the individual trials was  $0.31 \pm .04$  per cent. Ten of the moisture analyses were lower by the modified oil method, whereas

TABLE 1

*The moisture content of Cheddar cheese when determined by the steam oven method at 85 pounds pressure, by the olive oil method, and by the modified olive oil method*

Sample	Steam Pressure Oven Method	Olive oil method		Modified oil method	
No.	Moisture	Moisture	Difference from steam oven	Moisture	Difference from steam oven
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
1	37.75	36.60	- 1.15		
2	35.53	34.90	- 0.63		
3	34.52	34.31	- 0.21		
4	34.69	33.85	- 0.84		
5	34.53	34.14	- 0.39		
6	34.48	33.75	- 0.73		
7	34.85	34.03	- 0.82		
8	36.63	35.72	- 0.91		
9	30.69	29.99	- 0.70		
10	33.92	33.87	- 0.05		
11	31.73	30.29	- 1.44		
12	32.49	31.73	- 0.76		
13	44.62	44.02	- 0.60		
14	44.24	44.05	- 0.19		
15	44.08	43.50	- 0.58		
16	42.82	42.76	- 0.06		
17	41.94	41.96	+ 0.02		
18	43.81	43.77	- 0.04		
19	38.86	38.71	- 0.15		
20*	38.34			38.07	- 0.03
21	36.95	36.43	- 0.52	36.76	- 0.19
22	37.02	36.75	- 0.27	37.17	+ 0.15
23	33.78	32.78	- 1.00	33.10	- 0.68
24	33.83	33.54	- 0.29	33.12	- 0.71
25	34.44	34.02	- 0.42	34.48	+ 0.04
26	37.54	37.28	- 0.26	37.45	- 0.09
27*	38.80			38.89	+ 0.09
28	40.86	40.28	- 0.58	40.84	+ 0.04
29*	40.75			40.60	- 0.15
30	35.10	34.76	- 0.34	34.83	- 0.27
31*	34.57			34.85	+ 0.28
32	36.37	36.46	+ 0.09		
33	39.52	40.24	+ 0.72	39.89	+ 0.37
34	35.41	35.23	- 0.18	34.84	- 0.57
35*	39.89			39.61	- 0.28
36*	40.95			41.91	+ 0.96
37	39.94	39.75	- 0.19	39.77	- 0.17
Average, 31 trials	37.19 ± .47	36.75 ± .49	0.49 ± .04**		
Average, 17 trials	37.51 ± .41			37.42 ± .45	0.31 ± .04**

\* Samples spattered when attempts were made to analyze them by the regular olive oil procedure.

\*\* Signs neglected in computing average.

7 were higher. As was observed in the previous paper, certain samples analyzed by the modified olive oil method could not be analyzed by the regular oil procedure due to spattering during heating which threw a portion of the material from the dish.

#### SUMMARY

A comparison was made between the steam oven method operating at approximately 85 pounds pressure; the regular olive oil method, in which 5 grams of cheese are placed in olive oil and heated to dryness directly over a small gas flame; and the modified olive oil in which sodium chloride is added to the cheese-olive oil mixture to prevent spattering and sticking of the cheese. The results showed the olive oil method to give values averaging approximately 0.35 per cent lower than the oven method, whereas the salt-olive oil procedure gave results which averaged within 0.1 per cent of those secured by the steam oven.

Either of these oil methods appear to give results which, on the basis of these comparisons with the steam pressure oven method, would be accurate enough for all practical purposes. Further, the oil methods have the distinct advantage of requiring less time than the oven procedure, since the test may be completed within 20 to 30 minutes, especially when the modified procedure is followed.

The modified oil method has greater applicability than the regular olive oil method since (a) it permits more rapid heating and drying due to the fact that the particles of cheese do not lump together, (b) it prevents the cheese from sticking to the bottom of the pan during the heating process, and (c) it has permitted practically all of the Cheddar cheese samples thus far encountered to be analyzed without experiencing the difficulty with spattering which occurs frequently with the regular oil method. However, during some recent trials, two samples of cheese did not lend themselves to moisture determination by the modified oil method because of spattering. Both of these samples were abnormally high in moisture which may account for their behavior.

Although this and the previous paper have dealt with the use of olive oil in the open flame test, other oils with higher volatilization points may be superior to the olive oil for this method of moisture analysis. It was observed in the trials reported that some volatilization of the olive oil did occur during heating, resulting in the formation of a disagreeable odor. This slight volatilization, however, did not appear to have any appreciable influence on the moisture results. Several trials have since been carried out using cottonseed oil, and indications are that this oil may be superior to olive oil for the moisture test. It has a higher volatilization point and is considerably less expensive. Mineral oil is less satisfactory since it requires a relatively low temperature for volatilization.

## ACKNOWLEDGMENT

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## THE KEEPING QUALITY OF BUTTERS

### I. THE RATES OF DETERIORATION OF BUTTERS MADE FROM CREAMS OF DIFFERENT ACIDITIES AND STORED AT VARIOUS TEMPERATURES\*

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The question of the effect of the acidity of cream upon the keeping quality of its butter has been the subject of numerous discussions and investigations. While it is generally agreed that butter made from sweet cream keeps better in storage than butter made from acid cream, there seems to be some differences of opinion on this subject when dealing with a product made from cream known as "low-acid cream." Furthermore, all acid cream butters do not exhibit the same rate of deterioration in storage, but vary in this respect with the degree of acidity of the cream from which they were prepared.

In reports of the Iowa Experiment Station (1) (2) (1890 and 1892) work is reported which showed that butter made from sweet cream kept better than did butter made from sour cream. In each instance raw cream was used. Studies begun in 1905 by the U. S. Department of Agriculture (3) (4) (5) of the influence of the acidity of cream upon the keeping quality of butter, established the fact that butter made from unripened pasteurized sweet cream maintained its fine quality to a high degree during at least 8 months of storage at 0° C.

The deterioration of butters made from creams of different acidities was studied by White, Trimble and Wilson (6), who found that butters made from creams with acidities of from 0.15 per cent to 0.31 per cent kept well in storage at 0° F. for 8 months. After 12 months at 0° F. butters from creams of from 0.15 to 0.25 per cent acidity had deteriorated less than those made from creams of 0.28 to 0.31 per cent acidity, and the latter had deteriorated less than those made from creams of higher acidities. They found also that ripening cream with a lactic culture, even to relatively low acidities improved the score of the fresh butter therefrom, but the improvement was usually lost in storage. The deterioration was judged entirely by loss of score. The results upon the butters from cultured cream confirmed the conclusions of Mortensen (1922) (7) that ripened cream butter received a higher commercial score than did sweet cream butter when fresh but that sweet cream butter kept better in storage. This author states further (1936) (8):

"Research on cream ripening was continued for several years and we recommend at present that the acidity shall not exceed .36 per cent in the

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serum. That represents an acidity in a 30 per cent cream of  $.70 \times .36$  or .25 per cent. Whether this figure will be changed somewhat in the future will depend on the research of the next few years; it is my belief that it is about right. By applying the proper system of ripening, this degree of acidity will produce flavor and keeping qualities superior to those of sweet cream butter."

In order to determine accurately the relative keeping quality of butters from creams of different acidities their rates of deterioration over a relatively long storage period must be known. This is especially true of butters made from low acid creams whose rates of deterioration are slow in the initial periods of storage and accelerate with increased time of storage. In the present work, therefore, samples of each butter were, except in a few cases, scored and tested at regular intervals until the respective butter was declared unfit for consumption.

Though "score" has proved invaluable as an index of the quality of butter it is not especially valuable as a test by which chemical changes of butter may be detected or followed with accuracy. It is rather an opinion of quality based upon a number of factors and has no definite meaning except as it relates to edibility of the product. In the present work the "score" has been supplemented with chemical tests which have proved of value in the study of the deterioration of fats and oils. These tests have furnished data of a quantitative nature by means of which the rate of deterioration through oxidation changes have been accurately expressed and the relative value of the score method and other methods determined. The data obtained furnished also some information upon one of the major problems under consideration; namely, a method which would indicate early in the storage period the susceptibility of butterfat to chemical change and could therefore be used to predict keeping quality.

#### EXPERIMENTAL

Fresh creams of 0.12 to 0.14 per cent acidity were pasteurized and churned to furnish the control samples of butter. Other samples were pasteurized, brought to an acidity of approximately 0.20 per cent, others to acidities of approximately 0.30 and 0.40 per cent, respectively, and then churned. The butters from each churning were divided into five lots and samples of these were stored at each of five different temperatures. The chosen temperatures of storage were 20° C., 10° C., 0° C., -10° C., and -17° C., respectively. The samples stored at -17° C. were tested and scored at 90-day intervals, those at -10° C. at 80-day intervals, etc., as indicated in Table I.

The tests chosen to indicate the rate of deterioration were score, peroxide value, time of bleaching at 42° C., and two dye reduction tests. The last four of these tests are definitely related to oxidation, a reaction which our studies on fats and oils have led us to believe is directly and indirectly an underlying cause for many of the off flavors of butter. Because of many

TABLE 1  
The averages of values obtained in the different tests, upon butters stored for varying periods of time at different temperatures

Average acidity of cream (% lactic)	Number of samples	Days in storage	Peroxide value (M. mols per k)	Bleaching time at 42° C. (Days)	Average score	Days in storage	Peroxide value (M. mols per k)	Bleaching time at 42° C. (Days)	Average score	Days in storage	Peroxide value (M. mols per k)	Bleaching time at 42° C. (Days)	Average score
Storage at 20° C.													
.13	5	0	0	88	91.7	20	0.18	89.5	52	0.88	66	88.7	
.19	8	0	0	86	91.7	20	.09	89.8	52	.73	71	89.0	
.31	5	0	0	88	92.0	20	.62	89.0	52	1.62	31	88.2	
.41	4	0	0	70	91.25	20	1.25	86.6	52	7.41	13	86.3	
Storage at 10° C.													
.13	5	0	0	88	91.7	35	0.28	90	91.1	72	0.63	79	90.0
.19	8	0	0	86	91.7	35	.19	102	89.8	72	.61	71	89.7
.31	5	0	0	88	92.0	35	1.13	56	88.8	72	1.22	50	88.8
.41	4	0	0	70	91.25	35	1.96	30	86.9	72	2.92	20	86.5
Storage at 0° C.													
.13	5	0	0	88	91.7	70	0.23	84	91.5	140	0.41	68	88.9
.19	8	0	0	86	91.7	70	.17	105	92.0	140	.70	69	89.6
.31	5	0	0	88	92.0	70	.74	74	90.8	140	1.32	60	87.4
.41	4	0	0	70	91.25	70	1.44	44	88.6	140	3.39	38	87.0
Storage at -10° C.													
.13	5	0	0	88	91.7	81	0.00	77	92.0	160	0.15	70	91.0
.19	8	0	0	86	91.7	81	.08	76	91.5	160	.54	58	90.6
.31	5	0	0	88	92.0	81	.17	81	91.4	160	1.05	51	90.0
.41	4	0	0	70	91.25	81	.93	43	89.4	160	1.84	44	90.4
Storage at -17° C.													
.13	5	0	0	88	91.7	90	0.00	92	91.7	180	0.00	59	90.9
.19	8	0	0	86	91.7	90	.00	97	91.6	180	.22	65	90.8
.31	5	0	0	88	92.0	90	.17	55	90.9	180	.53	55	90.2
.41	4	0	0	70	91.25	90	.68	39	88.9	180	1.58	39	89.4
Storage at -20° C.													
.13	5	0	0	88	91.7	360	0.00	92	91.7	270	0.87	69	88.7
.19	8	0	0	86	91.7	360	.00	97	91.6	270	.88	73	89.1
.31	5	0	0	88	92.0	360	.17	55	90.9	270	1.06	66	88.4
.41	4	0	0	70	91.25	360	.68	39	88.9	270	1.70	38	87.4

difficulties encountered in the application of the dye reduction tests, the data relating to this phase of the work will not be presented here.

In order to obtain a true value for the rate of deterioration and one that would be comparable for the different butters, especially for those held at

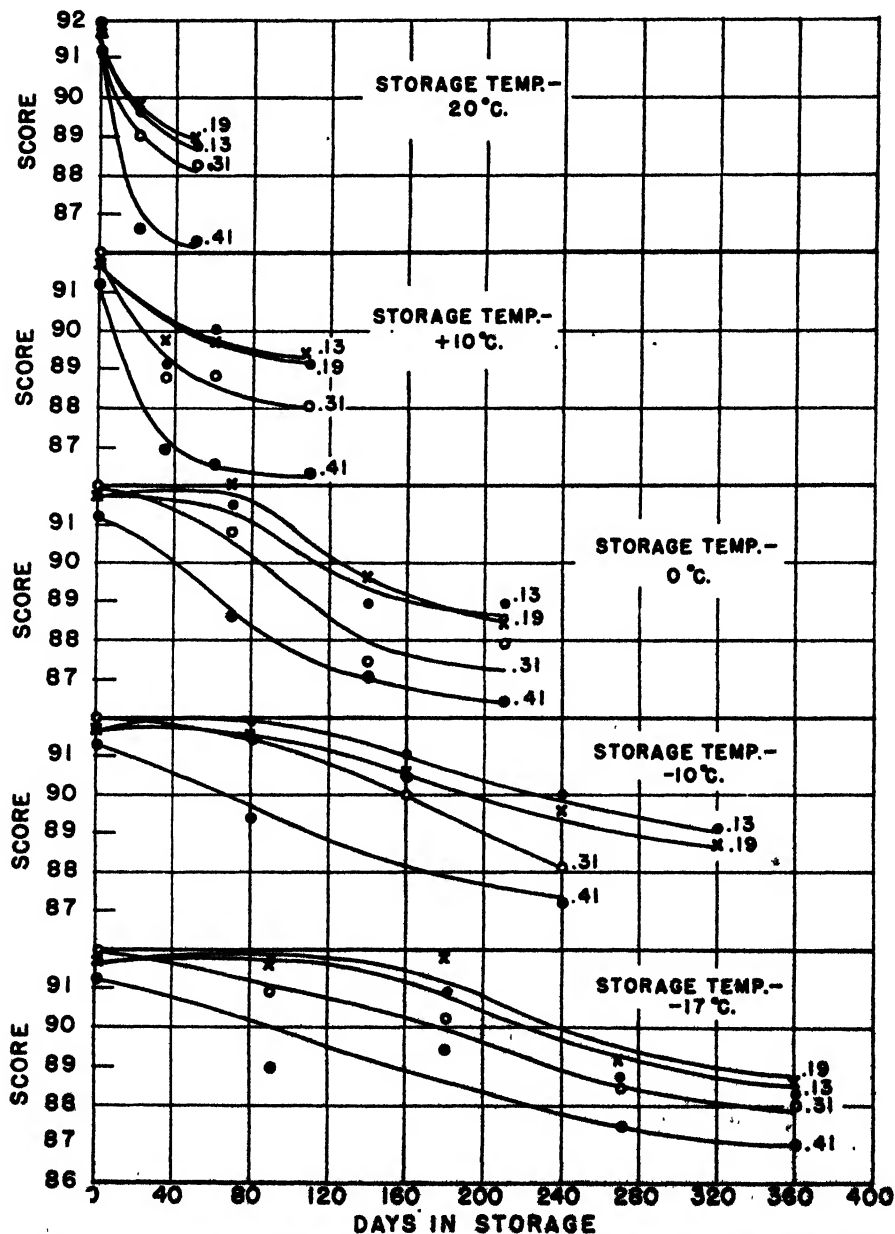


Fig. 1. The rates of loss of score of butters made from creams of different average acidities and stored at various temperatures.

TABLE 2  
Scores and remarks of scorers on the various samples of butter stored for various periods of time at  $-17^{\circ}\text{C}$ .

Sample No.	Acidity % Lactic	Initial		At 90 days		At 180 days		At 270 days		At 360 days	
		Score	Remarks	Score	Remarks	Score	Remarks	Score	Remarks	Score	Remarks
1-A	.13	92.0	Clean, flat	91.5	Stale	91.5		90.5		88.0	
2-A	.13	92.0	Sl. coarse	92.0		91.0		88.0		88.0	
3-A	.13	92.0		92.0		90.0		89.5	Stale, oily		
4-A	.14	91.5	Sl. oily	90.0		91.0		88.0			
5-A	.12	91.0	Oily, foreign	91.5		91.0		87.5			
1-B	.185	91.5		91.0		91.0		90.0		89.0	
2-B	.18	92.0		92.0		90.0		88.0			
3-B	.195	92.0	Clean, flat	92.5		91.5		88.0			
4-B	.18	92.0		92.0		91.0		88.0			
5-B	.20	92.5	Aroma, flavor	91.5		90.0		89.5	Stale, oily	88.0	
6-B	.195	92.0		92.0		90.0		89.5		89.0	
7-B	.20	91.0	Over-ripe, str. flavor	91.0	Sl. oily	91.5		90.0		89.0	
8-B	.20	91.0	Oily	91.0		91.0	Oily	90.0		88.5	
1-C	.30	92.5	Starter aroma & flavor	90.0	Metallic	91.0		88.0			
2-C	.36	92.0		90.0		90.0		90.0		88.0	
3-C	.30	92.0	Over-ripe flavor, aroma	91.4	Sl. oily	90.0	Sl. oily	89.0	Stale	88.0	
4-C	.30	92.0		91.5		91.0		88.0			
5-C	.30	91.5		92.0		89.0		87.0			
1-D	.37	91.5	Sl. oily	89.5	Metallic	91.0		87.5			
2-D	.46	90.0	Oily, sl. metallic	88.0	Sl. metallic	88.0		86.0			
3-D	.43	92.0	Str. aroma, sl. oily	88.0		88.5		87.0	Fishy		
4-D	.40	91.5	Oily	90.0		90.0	Aged	87.0		87.0	

different temperatures of storage, the surface layer was discarded in each case before the samples to be tested were taken. After scoring, the remainder of each sample was melted at about  $50^{\circ}\text{C}$ . and the fat filtered. This fat was then used in the various tests.

The data obtained are given in the following tables and charts. The results upon each individual sample are of no particular interest, hence the averages for the samples in each particular group have been used, except in the matter of "remarks by the scorers," which of course does not lend itself to such treatment. The remarks of the scorers are given in tables 2, 3, 4 and

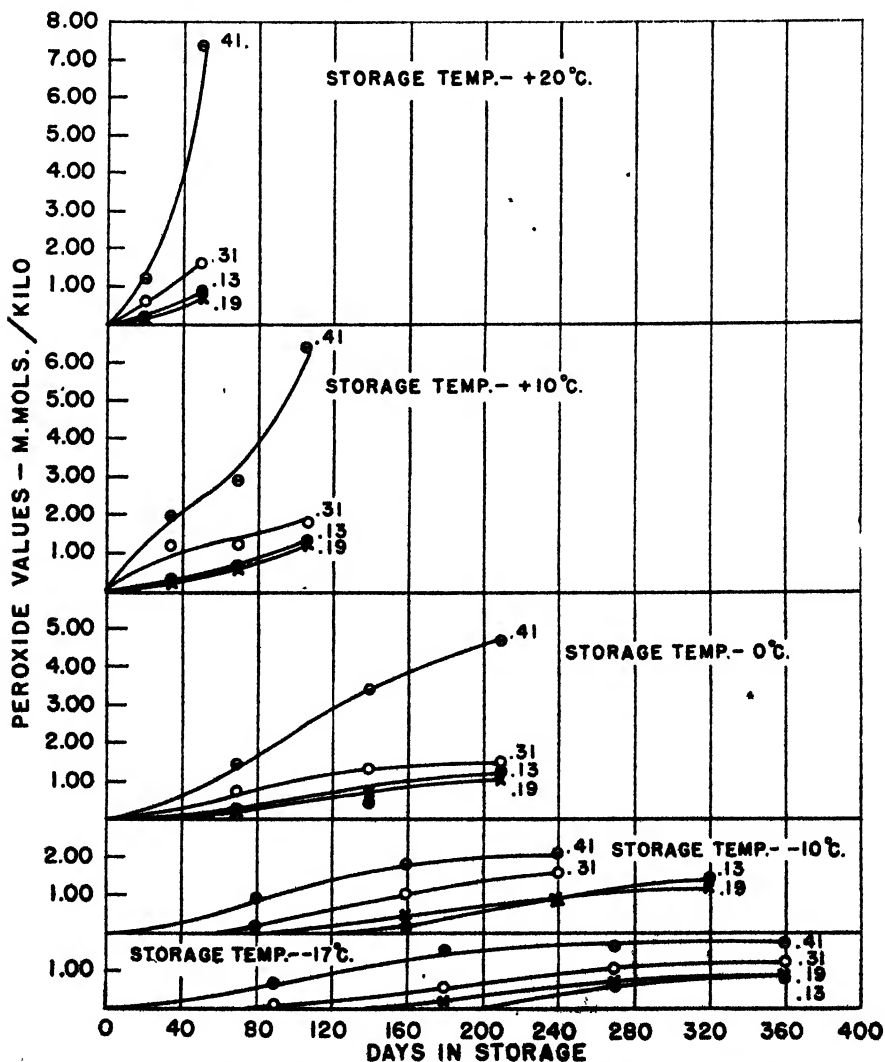


Fig. 2. The rates of peroxide formation in butters made from creams of different average acidities and stored at various temperatures.

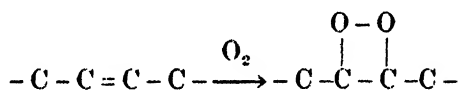
5. Their especial significance will be discussed later. In Table 1 are given the averages of the values obtained with each test upon the samples of butter in the various groups.

Since the periods of storage between tests varied with storage temperature, it is difficult to obtain a true comparison of the rates of variation in the values from this table. Such a comparison may be made with the aid of the following graphs. The values in Figure 1 indicate the relative rate of loss in score in butters from creams of different average acidity, when the butters are stored at the indicated temperatures.

The first marked difference is that of rate of loss in score by the samples of different acidities, at each temperature of storage; except in the case of butters made from creams of 0.13 and of 0.19 per cent average acidity.

The graphs indicate further that the rates of deterioration at the lower temperatures are not constant but progress very slowly for a period of time—the length of this phase being determined by the temperature of storage—then accelerate. No “period of induction” for the changes that take place were noted in those samples stored at 10° and 20° C.

The primary stage of oxidation of fats is the addition of oxygen to the unsaturated bonds of the unsaturated acid components, to form peroxides as follows:



These compounds are stable at ordinary temperatures in relatively low concentrations, and their amounts may be determined accurately through their ability to liberate iodine from potassium iodide in acid solution (9). Further oxidation of these compounds results in splitting of the acid with formation of compounds possessing tallowy flavors and odors. The rates of the formation of peroxides in the different butters are shown in Figure 2.

As in the case of the experiments in which the drop in score was used as a criterion of deterioration, those butters made from creams of 0.13 and 0.19 per cent average acidity show the same rate of deterioration when judged by the rate of peroxide formation. With increases in the acidity of the cream to 0.31 and 0.41 per cent, respectively, the rate of oxidation increased.

The results obtained with the bleaching test were not so consistent as those already given, but show the same order of the rates of deterioration for the different samples as was shown by the same samples of butter when judged by the loss in score or by the rate of peroxide formation.

Of the three tests discussed the peroxide value and the bleaching test relate definitely to oxidation. The score may reflect deterioration by oxidation, but is considered as a test which takes into consideration defects that may be the result of a number of different causes. Hence, to understand the type of deterioration which led to a loss in score in any particular sample,

TABLE 3  
Scores and remarks of scorers on the various samples of butter stored for various periods of time at  $-10^{\circ}\text{C}$ .

Sample No.	Acidity % Lactic	At 80 days		At 160 days		At 240 days		At 320 days	
		Score	Remarks	Score	Remarks	Score	Remarks	Score	Remarks
1-A	.13	92.0	...	92.0	Oily	90.5	...	88.5	Stale
2-A	.13	92.0	...	91.0	...	90.0	...	89.0	...
3-A	.13	92.0	...	91.0	...	91.0	...	91.0	...
4-A	.14	91.5	Very fine	90.0	...	...	Stale	89.0	...
5-A	.15	91.0	Fine, clean	90.5	...	89.0	Stale	88.0	...
1-B	.185	91.5	...	91.0	Sl. oily	90.0	Stale, oily	89.0	Stale
2-B	.18	91.5	Clean	90.0	...	89.0	Stale	87.0	...
3-B	.195	92.0	...	91.5	Sl. oily	90.0	...	88.5	...
4-B	.18	92.0	...	91.0	Oily	90.0	...	89.0	...
5-B	.20	92.0	...	91.0	...	90.0	...	90.5	...
6-B	.195	91.5	...	91.0	...	90.0	Stale	88.0	Stale, oily
7-B	.20	91.0	...	90.5	...	88.0	Stale, oily	88.0	...
8-B	.20	91.0	...	89.0	...	89.0	...	89.0	...
1-C	.30	91.0	...	91.0	...	88.0	...	...	...
2-C	.36	91.0	...	91.0	...	88.0	Stale	...	...
3-C	.30	91.0	...	88.0	Cooked, curdy, old	88.0	Fishy	...	...
4-C	.30	92.0	...	90.0	...	...	Stale	...	...
5-C	.30	92.0	Fine	90.0	Sl. aged	88.5	Stale, oily	...	...
1-D	.37	90.0	Sl. metallic	91.0	Oily	88.0	...	...	...
2-D	.46	88.0	Metallic	90.0	Stale, oily	87.0	...	...	...
3-D	.43	88.0	Metallic	90.0	Old, oily	...	Fishy	...	...
4-D	.40	91.5	Metallic	90.5	Aged	87.0	Fishy	...	...

TABLE 4  
*Scores and remarks of scorers on the various samples of butter stored for various periods of time at 0° C.*

Sample No.	Acidity % Lactic	At 70 days		At 140 days		At 210 days	
		Score	Remarks	Score	Remarks	Score	Remarks
1-A	.13	91.0	Stale	89.5	Aged	88.0	-----
2-A	.13	92.0	..	89.0	Sl. oily	88.0	..
3-A	.13	92.0	..	89.0	Storage flavor	90.0	..
4-A	.14	90.5	Sl. coarse	88.5	..	89.5	Stale
5-A	.12	92.0	Sl. coarse salt	88.5	..	..	Stale, oily
1-B	.185	92.0	..	91.0	Coarse	88.0	Stale, oily
2-B	.18	92.0	..	87.0	Cheesy	87.0	..
3-B	.195	92.0	..	89.0	Oily, sl. bitter	88.5	..
4-B	.18	92.0	..	88.0	Oily, sl. bitter	88.5	..
5-B	.20	92.0	..	89.0	Oily	90.5	..
6-B	.195	92.0	..	91.0	..	88.0	Stale, oily
7-B	.20	92.0	..	90.0	Storage & sl. acid	89.0	..
8-B	.20	92.0	..	92.0	..	88.0	..
1-C	.30	91.0	Stale	87.0	Fishy, greasy	88.0	Stale, oily, sl.
2-C	.36	91.0	Starter flavor	87.5	Fishy	87.5	metallic
3-C	.30	91.5	..	88.0	Storage, sl. acid	88.0	Stale
4-C	.30	89.0	S. metallic, soapy	88.5	..	89.0	..
5-C	.30	91.5	..	87.0	Cheesy	87.0	..
1-D	.37	90.0	S. metallic	88.0	Oily, sl. metallic	87.0	Bleached
2-D	.46	87.0	Fishy	85.0	Bitter, old, lardy, rancid	84.0	..
3-D	.43	87.0	Fishy	87.0	Old, rancid	87.0	Fishy
4-D	.40	90.0	Sl. metallic	87.0	Fishy	87.0	Stale, oily, sl. metallic

TABLE 5  
Scores and remarks of scorers on the various samples of butter stored for varying periods of time at 10 C.

Sample No.	Acidity % Lactic	At 35 days		At 72 days		At 108 days		At 140 days	
		Score	Remarks	Score	Remarks	Score	Remarks	Score	Remarks
1-A	.13	88.5	Cheesy	89.0	Stale, musty	87.0	Moldy	87.0	
2-A	.13	89.0	Stale	89.0	Stale, musty	89.0		89.0	
3-A	.13	90.0	Storage, oily	90.0	Stale	90.0	Stale	89.0	Stale
4-A	.14	..	..	91.0		90.5		90.0	
5-A	.12	..	..	90.0		89.0	Stale	89.0	
1-B	.185	90.0	Storage	90.0	Stale	90.0	Stale	89.0	Stale
2-B	.18	..	..	89.5	Musty	89.5	Cheesy	89.5	Stale
3-B	.195	88.0	Sl. cheesy, old	89.0	Stale, musty	88.0	Stale, sl. moldy	89.0	
4-B	.18	90.0	..	89.0	Stale, musty	89.0		89.0	
5-B	.20	90.5	Old, coarse	89.0	Stale	89.0	Stale	89.0	
6-B	.195	90.0	Old, oily	90.0	"	90.0	Stale	89.0	Stale
7-B	.20	90.0	Sl. oily	90.0	"	90.0		89.0	Stale
8-B	.20	90.0	Oily	90.0	"	89.5		89.0	Stale
1-C	.30	89.0	Stale, sl. metallic	88.5	Tallowy	87.0	Fishy	87.0	
2-C	.36	89.0	Sl. fishy	88.5	Metallic	88.0	Stale, oily	88.0	
3-C	.30	88.5	Old, oily	89.0	Stale	88.0	Stale, oily	88.0	
4-C	.30	..	..	90.0	Stale	90.0		89.5	
5-C	.30	..	..	88.0	Stale, oily	88.0	Cheesy, stale, oily	89.5	
1-D	.37	88.0	Stale, oily	87.0	Metallic	86.0	Fishy	86.0	
2-D	.46	85.0	Fishy, bleached	85.0	Fishy	85.0	Bleached, stale, oily	85.0	
3-D	.43	87.0	Fishy	86.0	Bleached, fishy	86.0	Bleached, stale, oily	86.0	
4-D	.40	87.5	Stale	88.0	Metallic	88.0	Stale, oily	88.0	

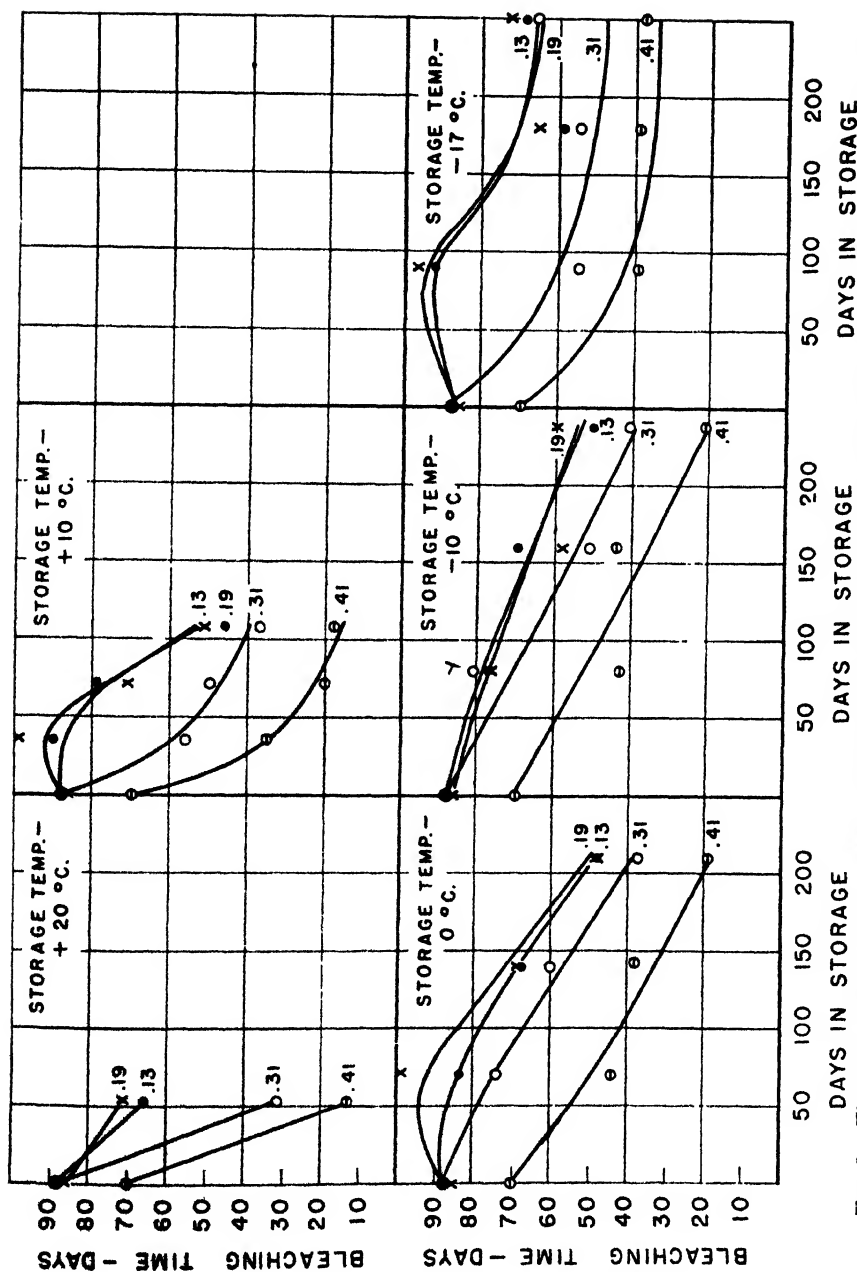


Fig. 3. The time necessary at 42° C. for bleaching of butters made from creams of different average acidities and stored at various temperatures.

the remarks of the scorers are of especial value. These are given in Tables 2, 3, 4, and 5.

A survey of these tables indicates little or no difference between the butters made from creams of 0.13 and 0.19 per cent acidity in the type of deterioration during storage. In none of these samples did the scorers note a metallic, tallowy, or fishy flavor, although some samples were stored for 360 days at  $-17^{\circ}\text{C}$ . Samples of butter made from 0.30 and 0.40 per cent acid cream possessed metallic flavors and fishy flavors relatively early in the storage period, the time of their occurrence depending upon the temperature of storage and the acidity of the creams from which they were made. Metallic and fishy flavors occurred more frequently and earlier in the storage period in the butters made from creams of 0.40 per cent acidity than in that made from 0.30 per cent acidity.

The relative efficiency of the different temperatures in promoting keeping quality of the different butters may be determined from the values in Table 1. The relative efficiencies have been shown graphically by plotting the time of storage in days required by each butter at each storage temperature, to result in a loss of two points in score.

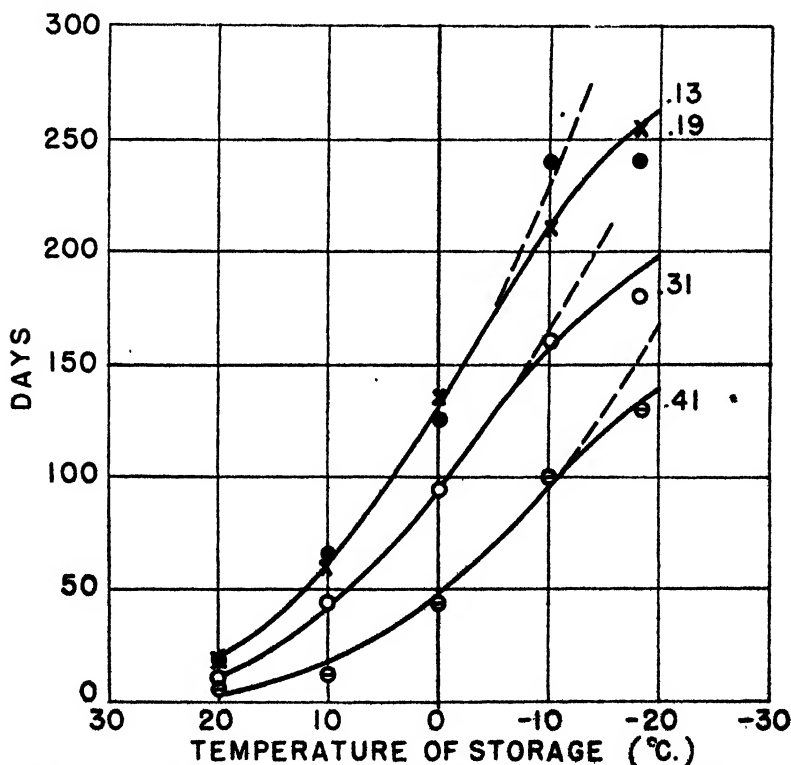


FIG. 4. The number of days of storage necessary at the indicated temperatures to result in a loss of two points in the score of butters made from creams of different acidities.

A decrease of the temperature from  $-10^{\circ}\text{C.}$  to  $-17^{\circ}\text{C.}$  does not seem to decrease the susceptibility of butters to deterioration to so great an extent as do similar decreases in the higher temperature ranges.

The graphs indicate also in a general manner the relative keeping quality of butters made from creams of different acidities.

#### DISCUSSION

Butters made from creams of different acidities grouped themselves similarly when their keeping quality was judged by any of the three tests used. In each case practically no difference was noted in the values obtained upon butters from creams of 0.13 and 0.19 per cent acidity at any of the chosen temperatures of storage. In other words, the rates of deterioration of sweet cream butters and of butters made from creams of less than 0.20 per cent acidity, developed by pure cultures added to sweet creams, were practically identical. Whether or not butters made from creams of acidities slightly greater than 0.20 per cent, possess a similar rate is not indicated by the data. Butters made from cream of an acidity of 0.30 per cent or more were found to be inferior in keeping quality to those made from sweet cream.

From the standpoint of reliability in judging deterioration, the score method will probably always be preferred, because of its direct relationship to edibility. However, it may not be directly related to any one type of chemical change and lacks in exact quantitative aspects, especially after the initial deterioration changes have taken place. Below a score of 89 the drop in score does not seem to correlate directly with the magnitude of the chemical changes that take place. This is noted especially in the relationship between scores and peroxide values of the samples stored at  $20^{\circ}$ ,  $10^{\circ}$  and  $0^{\circ}$  (see Figs. 1 and 2).

The general similarity in the rates of deterioration, determined by the loss in score and by the development of peroxide, throughout the period of storage, in spite of the fact that in scoring all off flavors and odors are taken into consideration, seems to verify the hypothesis that there is a direct relationship between rate of oxidation and loss in score, or stated more directly, the oxidation reaction seems to underlie the various changes that are responsible for the loss in score.

A measure of the rate of oxidation of a butterfat seems, therefore, to be a direct measure of the rate of deterioration even though the direct end product of this reaction—tallowiness—may not be noted in the scoring.

Fishy flavors and odors in butter have been shown to be caused by an acid medium and especially when accompanied by oxidation promoted by metals (3) (10). The present results verify the conclusion that acids promote this type of deterioration and show also that spontaneous autoxidation will aid its development. However, it appears that an acid medium is the major factor concerned, for the amount of peroxides developed in sweet cream butters was

often equal to or greater at the end of the storage period than the amounts in the acid cream butters when the latter possessed a fishy flavor, yet none of the butters made from cream of an acidity of 0.20 per cent or less developed a fishy flavor. The exact rôle that oxidation may play in the development of fishy flavors is not clear.

The efficiency of different temperatures of storage in promoting keeping quality is shown in Figures 1, 2, and 3. Their relative value is shown in Fig. 4, which seems to indicate that a drop in storage temperature from  $-10^{\circ}$  C. to  $-17^{\circ}$  C. does not result in an increase in keeping quality proportionate to that observed for a similar lowering at a higher temperature range.

#### ACKNOWLEDGMENT

Mr. C. S. Trimble of this Bureau aided Mr. White in the scoring of the butter samples and we wish to express our appreciation for his assistance.

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# SPECIFIC HEAT AND THE PHYSICAL STATE OF THE FAT IN CREAM

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## INTRODUCTION

The physical state and alterations in the physical state of the fat in the globules of milk and cream offer the most reasonable explanation for the profound effect of temperature on creaming, cream viscosity, foaming, churning, lipase activity, and surface tension. Many of these effects are reversible and are probably produced or influenced by the materials adsorbed on the surface of the globules when the fat is in different physical states. In order better to understand the influence of temperature on milk products, a knowledge of the physical state and alterations in the physical state of the fat in the globules is necessary. Information obtained by a study of milk-fat in mass cannot be used as indicating accurately the physical state of the fat dispersed as globules. Seeding occurring in a mass of fat will influence the crystallization of the whole mass, but in fat dispersed as globules seeding will affect only the fat in the globule in which the crystal happens to form, and will exert no direct effect on the fat in other globules. This leads to a much slower attainment of equilibrium. Furthermore the crystals are larger in fat in mass, as compared with fat in globules, and this influences the rate of solution or melting on heating. Crystallization in both fat in mass and in globules is influenced by the rate of cooling.

A study was undertaken to gain information as to the physical state of the fat in globules at different temperatures and the rate of attainment of a constant physical state at the different temperatures. Determinations of specific heat were used as one method of following the alterations in the state of the fat in the fat globules.

Chevreul (3) observed that the solidification of milkfat was accompanied by the evolution of heat. Fleischmann (5) (6), Fjord (4), Landois (9), Chanoz and Vaillant (2), Schnorf (10), Hammer and Johnson (7), and Bowen (1) have reported specific heat values for milk and cream. The effect of fat has been studied more specifically by Fleischmann (6), Hammer and Johnson (7), and Bowen (1), the latter reporting values obtained by the U. S. Bureau of Standards. There is no assurance that the fat was in equilibrium at the different temperatures used. The use of an ordinary calorimeter involves difficulties when used to determine the specific heat of milk and cream at temperatures within crystallization range, because considerable time is required to attain the equilibrium state, and during this

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time the heat exchange between the calorimeter and its surroundings makes up an ever increasing part of the total heat measured.

## EXPERIMENTAL

### *Method*

The method of mixtures was used, since it avoids the errors due to large values for heat exchange with the surroundings, and has a reputation for high accuracy. Quart thermos bottles were used as containers. Warm water from one bottle was poured into colder cream or milk contained in another. The thermometers used were graduated to  $0.1^{\circ}$  C. and were calibrated against each other over the range used—from 0 to  $75^{\circ}$  C. All readings were made with the aid of a magnifying glass and were estimated to  $0.02^{\circ}$  C. The errors and heat losses in the procedure were studied, and all equipment and procedures were thoroughly standardized. The heat "loss" to the container and during the manipulation was found to be given by the following equation:

$$\text{Loss calories} = K_1(t_f - t_i) + K_2(t_w - t_r)$$

where  $K_1$  and  $K_2$  are constants representing the heat equivalent of the apparatus and heat lost in pouring, respectively,

$t_f$  = final temperature of cream-water mixture,

$t_i$  = initial temperature of the cream,

$t_w$  = the temperature of the warm water before pouring,

and  $t_r$  = the temperature of the room.

It was found by experiment that within the normal limits, the relative humidity of the room exerted an effect so small that it could be disregarded. The constants were determined by calibration with water, the room temperatures varying from  $23$  to  $28^{\circ}$  C., cold water varying from  $5$  to  $39^{\circ}$  C., and warm water varying from  $21$  to  $66^{\circ}$  C. The constants were calculated by the method of least squares, and for a typical pair of thermos bottles gave the equation:

$$\text{Loss calories} = 25.35(t_f - t_i) + 3.38(t_w - t_r)$$

About 150 grams of cream and 250 grams of water were used. The amounts were determined by weighing. The specific heat of water was taken as unity, and after correcting for the heat loss, the specific heat of the cream was calculated.

### *Results*

The specific heat of skimmilk was determined both by the method of mixtures and by means of the conventional calorimeter in which heat was produced by an electric heater immersed in the milk. Within rather narrow limits the same values were obtained by both methods. This indicates that the heat of dilution involved in the method of mixtures is negligible. The average value was found to be 0.943 with only slight deviations for the

various samples over the temperature range studied. The specific heat of the fat in the cream was calculated from the specific heat of the skim milk, the specific heat and the fat content of the cream.

Cream of about 40 per cent fat content obtained from mixed milk was first warmed to 45° C. and was then cooled rapidly by immersing the container in water the temperature of which was about 5° C. below the final temperature desired for the cream. As soon as the desired temperature of the cream was reached, the temperature of the water bath was adjusted to the temperature of the cream, and an aliquot of the cream was removed at once and its specific heat determined. Determinations of specific heat were continued at intervals until it was evident that a constant value for that particular temperature had been reached. Before introducing the cream into the bottle, the temperature of the thermos bottle was adjusted to that of the cream by means of water. The temperature of the warm water which was poured into the cream was so adjusted that the resulting mixture would

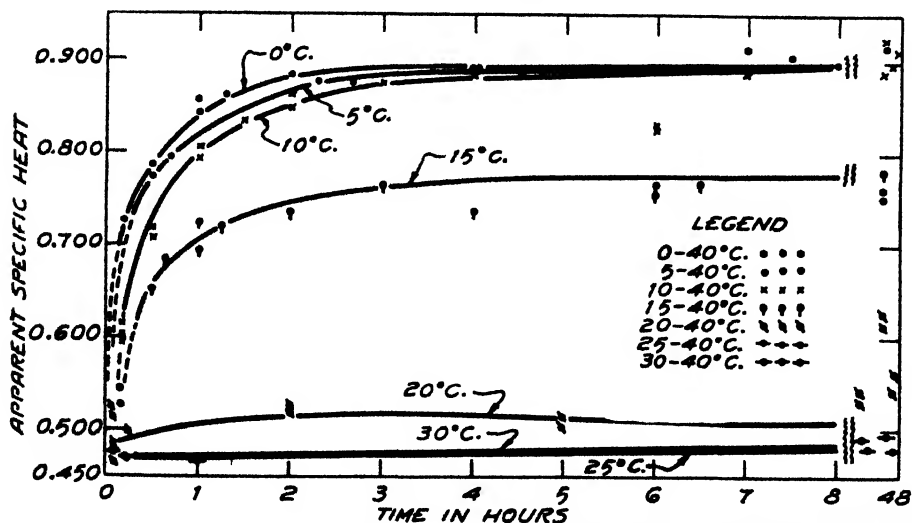


FIG. 1. The apparent specific heat of milkfat in cream held at various temperatures for varying lengths of time.

have a temperature of 40° C. or slightly above. In this way values were obtained for the heat required to warm to 40° C. cream having original temperatures ranging from 0 to 30° C. The specific heat values thus obtained are the average specific heats for the temperature ranges. The specific heat values attributable to the fat alone are presented graphically in Figure 1. This figure indicates that if the cream is cooled rapidly enough the fat at rather low temperatures may for a short time still be liquid and have the specific heat of liquid fat. Crystallization soon begins, however, and continues for about 4 hours, since the specific heat increases for a period of approximately 4 hours. After this time the change is slight.

The effect of season and feed of the cow on the hardness of the fat has been studied by Hunziker, Spitzer and Mills (8) and others. The fat is generally less hard on pasture than on dry feeding. Figure 2 shows that there

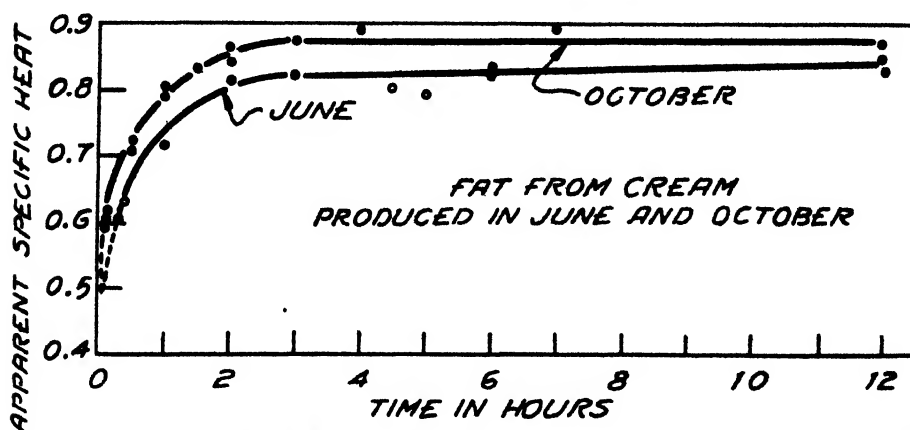


FIG. 2. The apparent specific heat of milkfat in cream produced in the month of June and in October. Cream warmed from 10° to 40° C.

is also a difference in specific heat of the fat in the temperature range in which crystallization of fat occurs.

Aliquots of cream, one at equilibrium at 15° C., the other at equilibrium at 20° C., differ in the number of calories required to warm the cream from equilibrium at 15 to equilibrium at 20° C. by the difference in the amount of heat required to raise each to 40° C., a temperature at which the fat is liquid. Using this procedure, the heat required to raise milkfat from successive equilibrium states at 5° C. intervals in temperature was calculated. In Table I are recorded the average equilibrium specific heat values of the fat calculated

TABLE 1

*Apparent specific heat of fat in cream for each 5° C. interval ranging from 0 to 40° C.  
Milk produced in October and November*

Temp. range	Tempera- ture dif- ference	Apparent sp. heat	Heat required	Heat required 5° C. interval	Average sp. heat for interval	Tempera- ture interval
°C.	°C.		calories	calories		°C.
30-40 ...	10	0.475	4.75		0.475	30-40
25-40 ...	15	0.475	7.13	2.38	0.475	25-30
20-40 ...	20	0.50	10.00	2.87	0.575	20-25
15-40 ...	25	0.77	19.25	9.25	1.85	15-20
10-40 .....	30	0.88	26.40	7.15	1.43	10-15
5-40 .....	35	0.89	31.15	4.75	0.95	5-10
0-40 .....	40	0.89	35.60	4.45	0.89	0- 5

from the calories required to warm to 40° C. cream held at a series of temperatures. The total number of calories required to warm the fat through the given temperature range was then calculated, and by difference the increments in calories for each 5 degrees. In the next to the last column the average specific heat of the fat in each 5 degree range is given. This table indicates that the apparent specific heat of the fat varies from 0.475 for liquid fat to 1.85 for the temperature range between 15 and 20° C., the range in which the greatest change in physical state occurs. If smaller increments in the region of 15° C. had been taken, still higher values might be obtained. The data in this table are in general agreement with those of Fleischmann (6), Hammer and Johnson (7), and Bowen (1). The data from Table 1 were used to calculate the specific heat increments over the temperature range of from 0 to

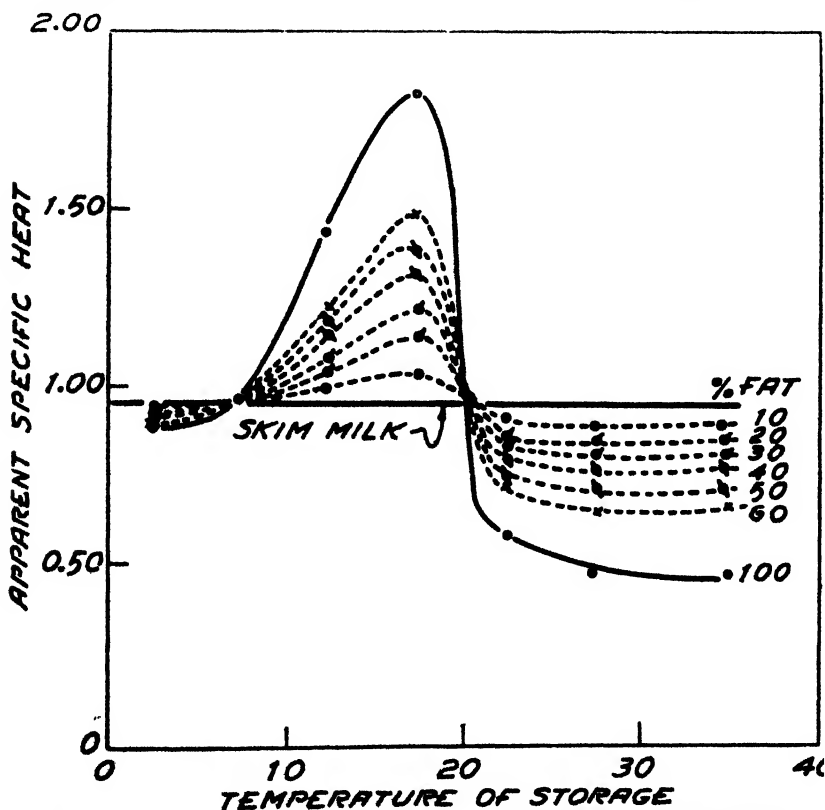


FIG 3. The calculated apparent specific heat of milkfat and of milk and cream, within each 5° C. interval in the 0–30° C. and in the 30–40° C. range. The calculations are based on values obtained on samples which had been stored at the various temperatures for 4 hours.

40° C. of milk and cream containing various percentages of fat, and the results are given in Table 2. The relationships are more readily visualized in Figure 3.

TABLE 2

*Specific heat of skim milk, cream, and milk fat globules for 5° intervals ranging from 0 to 40° C. milk produced in October and November*

Temperature interval °C.	Fat content per cent								
	0	5	10	20	30	40	50	60	100
0- 5	.943	.940	.937	.932	.927	.921	.916	.911	.890
5-10	.943	.943	.943	.944	.945	.945	.946	.947	.950
10-15	.943	.967	.991	1.040	1.089	1.138	1.186	1.235	1.43
15-20	.943	.988	1.033	1.124	1.215	1.305	1.396	1.487	1.85
20-25	.943	.924	.905	.869	.832	.795	.758	.722	.575
25-30	.943	.919	.895	.849	.802	.755	.708	.662	.475
30-40	.943	.919	.895	.849	.802	.755	.708	.662	.475

## DISCUSSION

The following melting point temperatures are typical of those given in the literature: tristearin 71° C., tripalmitin 65° C., trimyristin 55° C., and triolein -5° C. Stearic, palmitic, myristic and oleic are the principal acid components of milkfat. Milkfat may be considered as a solution which on cooling becomes supersaturated with respect to one or more of the mixed glycerides of which it is composed. The uncertainty and indistinctness of the so-called solidifying point would be expected, since a true solidifying point is not involved. The temperature at which a supersaturated solution begins to crystallize varies with the conditions, such as degree of supersaturation, agitation, seeding, etc. Furthermore, after crystallization starts the rate of crystallization of a solute from its supersaturated solution may vary greatly and crystallization may extend over a considerable period of time. The crystallization of milkfat is still further complicated because on cooling it probably becomes supersaturated with respect to more than one solute. Milkfat can readily be fractionated into one component which will not melt unless heated above 45° C., and into another which will not solidify when cooled to 5° C.

The so-called melting point of milkfat is not definite because we are really dealing with a temperature-solubility relationship. As the milkfat is warmed, the solid glycerides become more soluble in the liquid fractions, and an appreciable increase in solubility is noticed in the region of 15° C., but complete solution is usually not obtained until the fat is heated above 30° C. The rate at which the crystals dissolve is dependent upon their size, which in turn is dependent upon the rate and conditions of the previous cooling. These phenomena are all demonstrable with milkfat in mass.

On cooling, the lag in the formation of the solid phase in milkfat in the globule state is much greater than for fat in mass.

The alteration of the specific heat of cream with time was used as a method

for following the rate of formation of solid milkfat at the various temperatures. Below 20° C., the greater part of the crystallization is complete in about four hours. This is in agreement with some experiments carried out by Troy and Sharp (11), who showed that solidification of the fat globules, as indicated by resistance to pressure in the centrifuge, was not complete in two hours, but was complete in five hours, at 3° C.

#### CONCLUSIONS

1. The method of mixtures is a reliable method for the determination of specific heat of cream within the range in which a change in the relative amounts of crystalline and liquid fat occurs.

2. When cream is cooled to temperatures within the range of 0 to 20° C., the phase adjustment is nearly complete in about 4 hours, so far as this is indicated by the specific heat.

3. The average equilibrium specific heat for milk-fat in the globules for 5° C. temperature intervals is as follows: 0–5° C., 0.89; 5–10° C., 0.95; 10–15° C., 1.43; 15–20° C., 1.85; 20–25° C., 0.575; 25–40° C., 0.475; for fat in milk produced in October and November.

4. A variation in the specific heat of the milkfat with feed or season was shown.

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# BOUND WATER AND ITS RELATION TO SOME DAIRY PRODUCTS.

## II. FACTORS AFFECTING THE BOUND WATER CONTENT OF SOME DAIRY PRODUCTS\*

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Evidence that liquid dairy products contain bound water was presented in the first of this series of studies on bound water (1). There was also some indication that the bound  $\rightleftharpoons$  free water equilibrium could be changed readily by various treatments. Sayre (2) states that "Bound water does not exist in definite proportions relative to the solid material of the system, but as a ratio between bound water and free water. The ratio may be changed quickly by varying the temperature, acidity, surface energy, presence of electrolytes, pressure, etc."

Since the degree of hydration plays a rôle in giving colloidal stability to the product in question, it was thought advisable to determine some of the factors that influence the bound water content. If the product in question lacks colloidal stability, during or after manufacture, in many cases a partial precipitation of the proteins may occur. It can be readily seen that the nature of some of the defects that occur in ice cream, milk, evaporated milk, sterilized cream, etc., are usually associated with the ability of the milk proteins to bind water or to readsorb water after certain necessary treatments.

It has been known for a long time that certain effects are produced by various treatments of dairy products but just what causes some of these effects has never been definitely established. Therefore, a study of the factors affecting the bound water content of some dairy products was undertaken in the hope that some of these phenomena might be explained more fully.

### EXPERIMENTAL METHODS

The samples used, unless otherwise stated, were obtained from the same sources as previously mentioned (1). The experimental methods used were described before (1).

The data on aging, viscosity and protein stability given throughout this work will be dealt with in detail in the last paper of this series.

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\*\* The data presented in this paper are from a thesis submitted to the Graduate School of The Pennsylvania State College in partial fulfillment of the degree of Doctor of Philosophy, 1937.

*The Effect of pH on the Bound Water Content of Casein Sols*

A study was made of the effect of pH on the bound water content of casein sols. The casein used was pure casein (Pfanstiehl). It was dispersed with sodium bicarbonate to obtain the desired pH values. The effect of increased and decreased pH was determined, using a casein sol at a pH of 6.69 as the control. Table 1 gives a summary of the data obtained on the effect of pH on the bound water content of casein sols.

TABLE 1

*The effect of pH on the bound water content of casein sols*

Sample	Hours aged at 40° F.	Per cent solids	pH	Vis- cosity centi- poises	Per cent acid	Per cent bound water	Grams bound water per gram solids
1. Casein Sol (pH 5.83)	4	2.79	5.81	1.244	0.125	1.06	0.38
	24	2.79	5.83	1.283	0.125	1.24	0.44
2. Casein Sol (pH 6.69)	4	2.97	6.64	1.679	0.12	2.29	0.77
	24	2.97	6.69	1.718	0.12	2.42	0.81
3. Casein Sol (pH 7.36)	4	3.51	7.34	1.442	0.115	1.37	0.39
	24	3.51	7.36	1.540	0.115	1.77	0.50

The hydrogen ion concentration is probably one of the most important factors involved in the study of the bound water content of dairy products. It probably has a definite influence on the amount of water bound in dairy products since most proteins have well defined isoelectric points or zones.

The studies with casein sols in Table 1 show the importance of optimum pH in order to have maximum bound water content. Increasing or decreasing the pH from the "normal" brings about a decrease in the bound water content. A decrease in pH appears to be slightly more detrimental to the bound water content than an increase in pH.

*The Effect of pH on the Bound Water Content of Concentrated Milk Plasma*

The pH of normal ice cream mixes made from fresh products is usually between 6.2 and 6.4. Since pH plays such an important part in protein stability, it was thought that altering the pH of a concentrated milk plasma would affect the bound water content. Sodium bicarbonate was used to change the pH in the two samples. The pH of the fresh control was 6.22 which is normal for an ice cream mix. The results of these trials appear in Table 2.

Increasing the pH in the range studied has a marked effect on the water binding capacity of the concentrated milk plasma as can be seen from Table 2. A decrease in the bound water content resulted when the pH was increased to neutrality and slight alkalinity. It will be noted that although

TABLE 2

*The effect of increasing the pH on the bound water content of concentrated milk plasma*

Sample	Hours aged at 40° F.	Per cent solids	pH	Viscosity centipoises	Per cent acid	Alcohol no.	Per cent bound water	Grams bound water per gram solids
1. Conc. Milk Plasma pH 6.22	0	17.57	6.22	5.590	0.38	7.2	6.10	0.347
	4	17.57	6.24	6.183	0.375	7.3	6.76	0.384
	24	17.57	6.22	7.051	0.375	7.4	8.12	0.462
2. Conc. Milk Plasma pH 7.05	0	17.01	7.05	6.183		9.1	4.78	0.281
	4	17.01	7.08	5.293		8.9	5.60	0.323
	24	17.01	7.08	6.459		9.2	6.62	0.383
3. Conc. Milk Plasma pH 7.35	0	17.31	7.35	6.183		9.4	3.86	0.223
	4	17.31	7.37	6.755		9.2	4.77	0.275
	24	17.31	7.37	6.459		9.5	4.05	0.234

a decrease in bound water content resulted on increasing the pH, the protein stability as measured by the alcohol number increased.

Another series of samples was made and the effect of decreasing the pH on concentrated milk plasma was determined. Lactic acid was used to lower the pH of the samples.

The pH was decreased to 5.97 and 5.19 while the pH of the control was 6.31. The results of these mixes are recorded in Table 3.

TABLE 3

*The effect of decreasing the pH on the bound water content of concentrated milk plasma*

Sample	Hours aged at 40° F.	Per cent solids	pH	Viscosity centipoises	Per cent acid	Alcohol no.	Per cent bound water	Grams bound water per gram solids
1. Conc. Milk Plasma pH 6.31	0	14.75	6.31	5.293	0.300	7.8	2.37	0.161
	4	14.75	6.32	5.590	0.290	7.8	3.69	0.250
	24	14.75	6.32	5.886	0.290	7.7	5.37	0.364
2. Conc. Milk Plasma pH 5.97	0	15.14	5.97	4.701	0.575	3.0	0.31	0.020
	4	15.15	6.05	4.997	0.560	3.0	1.76	0.116
	24	15.14	6.05	5.590	0.560	3.0	3.08	0.203
3. Conc. Milk Plasma pH 5.19	0	15.14	5.19	4.701	0.590	2.0	0.11	0.007
	4	15.14	5.23	4.997	0.590	1.6	2.17	0.143
	24	15.14	5.23	4.997	0.595	1.5	1.72	0.113

It is very evident that a decrease in pH in the range studied decreases the bound water content of concentrated milk plasma. It is logical to believe that further decreases in pH toward the isoelectric zone would decrease the bound water content still more and that it should be at a minimum at the isoelectric point. It appears then that the phenomenon of precipitation is accompanied by a decrease in the bound water content.

It will be noted also that with the decrease in bound water content there was a decrease in viscosity and protein stability.

*The Effect of Concentration on the Bound Water Content of Casein Sols*

The casein used for this study was pure casein (Pfanstiel). It dispersed readily when the pH was adjusted with sodium bicarbonate. Sodium caseinate was undoubtedly formed which is more soluble than calcium caseinate and probably binds more water, but the results nevertheless indicate the effect of concentration on the bound water content of these casein sols. These results are recorded in Table 4.

TABLE 4

*The effect of concentration on the bound water content of casein sols*

Sample	Hours aged at 40° F.	Per cent solids	pH	Vis- cosity centi- poises	Per cent acid	Per cent bound water	Grams bound water per gram solids
1. Casein Sol (1.67%)	4	1.67	6.98	1.185	0.07	1.46	0.87
	24	1.67	6.97	1.244	0.07	1.53	0.92
2. Casein Sol (3.35%)	4	3.35	6.91	1.718	0.11	2.33	0.70
	24	3.35	6.89	1.758	0.11	2.50	0.75
3. Casein Sol (6.71%)	4	6.71	6.83	4.345	0.24	3.54	0.53
	24	6.71	6.81	4.444	0.24	4.42	0.66

The bound water content per gram of casein tends to diminish with the concentration of the casein sol. In general, it is known that a hydrophilic colloid will bind more water per gram of substance in dilute solution. Casein, then, acts like a hydrophilic colloid in this respect and seems to show that bound water is closely associated with concentration. Chrysler (3), working with kelp, has found similar results. Newton and Martin (4), experimenting on some colloidal sols, show that in most cases the water bound per gram of colloid tends to diminish with the concentration of the sols.

*The Effect of Varying Temperatures on the Bound Water Content of Concentrated Milk Plasma*

It is well known that the temperature of pasteurization of dairy products affects the physico-chemical properties of the product. It seems probable that varying temperatures would affect the bound water content. Certain high heat treatment is known to favor protein stability with alcohol but what effect it has on hydration has not been definitely known.

Three samples of concentrated milk plasma were compared. The control was heated to 145° F. for 30 minutes while another sample was heated to 175° F. for 5 minutes and the third sample was boiled for one minute. The data for this experiment are given in Table 5.

It will be seen in Table 5 that the higher heat treatment reduced the bound water content of the concentrated milk plasma. It would seem natural to expect that at high temperatures the colloidal micelles are partially

TABLE 5

*The effect of heat treatment on the bound water content of concentrated milk plasma*

Sample	Hours aged at 40° F.	Per cent solids	pH	Viscosity centipoises	Per cent acid	Alcohol no.	Per cent bound water	Grams bound water per gram solids
1. Heated 145° F. for 30 min.	0	13.80	6.38	3.970	0.29	7.9	4.01	0.291
	4	13.80	6.38	4.108	0.285	8.0	4.73	0.343
	24	13.98	6.38	3.239	0.285	8.0	2.56	0.183
2. Heated 175° F. for 5 min.	0	13.98	6.31	3.516	0.285	8.0	3.42	0.245
	4	13.98	6.36	3.832	0.28	8.1	4.41	0.315
	24	13.98	6.38	3.239	0.285	8.0	2.56	0.183
3. Boiled one minute	0	14.29	6.27	3.970	0.29	8.2	2.51	0.176
	4	14.29	6.33	4.108	0.29	8.3	3.28	0.230
	24	14.29	6.34	3.970	0.29	8.2	2.71	0.190

dehydrated and therefore a decrease in bound water content results. At the higher temperatures it will be seen that the protein stability as measured by the alcohol number increased but slightly. It will also be noted that the bound water content was lower after 24 hours of aging at 40° F. than after 4 hours of aging.

*The Effect of Heat on the Bound Water Content of the Fat Globule "Membrane" and Pure Milk Phospholipids*

Perlman (5) and Jack (6) have shown the effect of heat on the milk phospholipids. The latter worker believes that the action of heat is a denaturation process in removing the phospholipids from the surface of the fat globules. The writers have further observed that when cream containing approximately 50 per cent of butterfat is heated to 160° F. or higher, some "oiling off" of the fat will result if the cream is not stirred.

Fat globule "membrane" was prepared from washed cream as previously mentioned (1). To note the effect of heat on the bound water content of this material, samples were prepared and heated to various temperatures and aged 4 and 24 hours at 40° F. The samples used were: (1) unheated

TABLE 6

*The effect of heat on the bound water content of the fat globule "membrane"*

Sample	Hours aged at 40° F.	Per cent solids	pH	Per cent bound water	Grams bound water per gram solids
1. Unheated	4	2.52	6.32	1.55	0.615
	24	2.52	6.05	1.77	0.702
2. Heated to 143° F. for 30 min.	4	2.42	6.53	1.38	0.570
	24	2.42	6.43	1.50	0.619
3. Heated to 160° F. for 5 min.	4	2.49	6.63	0.90	0.361
	24	2.49	6.58	1.06	0.426

control; (2) heated to 143° F. for 30 minutes; and (3) heated to 160° F. for 5 minutes. The bound water studies and other observations are given in Table 6.

The effect of heat on the fat globule membrane is similar to that obtained for other fluid dairy products. A slight decrease of bound water content was obtained when the membrane material was heated to 143° F. for 30 minutes. A temperature of 160° F. for 5 minutes decreased the bound water content markedly.

Some observations on the appearance and odor of the material on heating indicate that some chemical change took place. The unheated control had the appearance of rich milk, had a creamy consistency and gave off no odor, while the sample heated to 143° F. for 30 minutes was lacking somewhat in creamy appearance and gave off a slight sulfur odor. The sample heated to 160° F. for 5 minutes was completely bleached in color, had a watery consistency and gave off a strong odor of sulfur similar to hydrogen sulfide.

An increase in pH and a decrease in acidity occurred on heating the fat globule "membrane." Possibly this reduction in acidity is due to the liberation of carbon dioxide. The viscosity studies show that there was a noticeable decrease on heating. This may have some bearing on the decrease in viscosity of cream on pasteurization.

The effect of heat on the bound water content of pure milk phospholipids was also studied. The pure milk phospholipids material was made as previously mentioned (1). The same temperatures of heating were used as in the previous experiment on the fat globule "membrane." Table 7 shows the results obtained in this experiment.

TABLE 7

*The effect of heat on the bound water content of pure milk phospholipids*

Sample	Hours aged at 40° F.	Per cent solids	pH	Vis- cosity centi- poises	Per cent acid	Per cent bound water	Grams bound water per gram solids
1. Unheated . . . . .	4	0.66	6.83	2.163	0.005	3.53	5.35
	24	0.66	6.82	2.204	0.005	3.95	5.98
2. Heated to 143° F. for	4	0.65	6.84	2.104	0.005	3.11	4.78
30 min. . . . .	24	0.65	6.82	2.102	0.005	3.34	5.14
3. Heated to 160° F. for	4	0.66	6.83	2.041	0.005	2.64	4.00
5 min. . . . .	24	0.66	6.82	2.081	0.005	2.92	4.42

The data in Table 7 show that heat lowers the bound water of pure milk phospholipids. It will be noted again with the temperatures used that the greatest reduction in bound water content occurs between 143° F. and 160° F.

The viscosity of the phospholipid sols decreased on heating to elevated

temperatures. From the results obtained it can be stated that certain high temperatures decrease the bound water content and viscosity of the fat globule "membrane" and pure milk phospholipids.

*The Effect of Pasteurization and Homogenization on the Bound Water Content of Twenty-Two Per Cent Cream*

A study with cream was undertaken to show the effect of pasteurization and homogenization on the bound water content. A sample of cream was divided into three lots as follows: (1) control of unheated cream; (2) cream heated to 143° F. for 30 minutes; and (3) cream homogenized with a pressure of 2000 pounds at a temperature of 143° F. These data are given in Table 8.

TABLE 8

*The effect of pasteurization and homogenization on the bound water content of cream*

Sample	Hours aged at 40° F.	Per cent solids	pH	Viscosity centipoises	Per cent acid	Per cent bound water	Grams bound water per gram solids
1. Raw Cream	4	28.53	6.64	7.285	0.15	4.12	0.144
2. Heated to 143° F. for 30 min.	4	28.77	6.66	6.326	0.14	4.01	0.139
3. Homogenized (2000 lbs.)	4	27.83	6.62	28.530	0.135	3.56	0.128

The pasteurization of cream is known to have a detrimental effect upon viscosity and this fact is borne out in Table 8. At the pasteurization temperature used, it is also evident that a small reduction in bound water content occurred.

Homogenization of cream at a temperature of 143° F. and a pressure of 2000 pounds markedly increases the viscosity and decreases the bound water content. The clumping of the fat globules apparently exerts an influence on the protein stability and bound water.

From this study it can be concluded that raw cream contains more bound water and has a higher viscosity than pasteurized cream. Homogenization decreases the bound water, but markedly increases the viscosity.

*The Effect of Homogenization on the Bound Water Content of "Ice Cream Mixes"*

Some of the effects produced by homogenization in milk and cream are: (1) stabilization of the fat emulsion; (2) destabilization of the protein; (3) increase in surface tension; (4) increase in titrable acidity; or (5) decrease in pH; and (6) increase in viscosity, normally thought to be due to the increase in volume of the disperse phase together with the adsorbed layer and the tendency of the fat to clump.

A "mix" was made containing 14.75 per cent of fat, 12.15 per cent serum solids and distilled water, the sugar and the gelatin being omitted. This "mix" was divided into four portions and treated as follows: (1) homogenized at 0 pounds pressure; (2) homogenized at 1500 pounds pressure; (3) homogenized at 3000 pounds pressure (single valve); and (4) homogenized at 3000 pounds and then at 700 pounds pressure (dual homogenization). The bound water determinations were made in the usual manner and the results are given in Table 9.

TABLE 9

*The effect of homogenization on the bound water content of "ice cream mixes"*

Mix pressure	Hours aged at 40° F.	Per cent solids	pH	Viscosity centipoises	Per cent acid	Alcohol no.	Per cent bound water	Grams bound water per gram solids
1. 0 lbs. ....	0	26.90	6.43	7.940	0.27	7.4	5.14	0.191
	4	26.90	6.43	9.125	0.27	7.4	5.56	0.207
	24	26.90	6.45	8.829	0.275	7.4	5.29	0.197
2. 1500 lbs.	0	27.01	6.39	12.938	0.275	7.2	2.43	0.090
	4	27.01	6.41	14.123	0.275	7.2	3.22	0.119
	24	27.01	6.42	14.715	0.280	7.2	3.79	0.140
3. 3000 lbs.	0	27.01	6.39	45.293	0.280	7.0	1.96	0.075
	4	27.01	6.41	52.641	0.285	7.0	2.23	0.083
	24	27.01	6.41	53.233	0.285	7.1	3.47	0.128
4. 3000 and 700 lbs.	0	27.01	6.42	10.499	0.275	7.3	5.65	0.209
	4	27.01	6.41	12.799	0.275	7.3	5.93	0.220
	24	27.01	6.41	12.409	0.280	7.4	6.18	0.229

It will be noted from Table 9 that the greater the homogenization pressure (single valve) the greater was the decrease in bound water content and protein stability. It is also apparent that as the viscosity increased due largely to fat clumping (sample 3) there was a decided reduction in bound water content. When the fat clumping and viscosity were decreased by the dual homogenization process (sample 4) a marked increase in bound water content was noted when compared to sample 3.

The fat clumping is undoubtedly largely responsible for the increased viscosity obtained on homogenization, and from the data presented it becomes apparent that the bound water content of these "ice cream mixes" decreases on increased fat clumping and that where dual homogenization is practiced an increase in bound water content and protein stability result.

#### *The Effect of Freezing Milk Plasma on the Bound Water Content*

Anderson and Pierce (7) found that after two or three months of storage in a frozen state milk proteins began to precipitate and there seemed to be a slight chemical reaction occurring.

Munkwitz, Berry and Boyer (8) show that freezing causes a partial precipitation of the milk solids; that albumin is precipitated in the greatest

amount, followed in order by lactose, total protein, ash, casein and fat; that with the exception of the fat, the amount of precipitation of the solids increases as the length of time of freezing increases; that freezing causes the fat globules to clump together, and become distorted and irregular in size and shape.

Under the usual conditions of freezing and thawing, the casein of milk, being hydrated, retains its normal degree of dispersion, but when the milk is held in the frozen state over long periods of time, various workers have found that the casein gradually precipitates. It is possible that its hydrophilic properties are changed during storage in a frozen state.

Webb and Hall (9) found that if whole milk is condensed  $2\frac{1}{2}$  to 3 times its normal concentration and is then frozen, it may be kept in storage at  $-13.3^{\circ}\text{C}$ . ( $8^{\circ}\text{F}$ .) or below and reconstituted at any time within about 6 weeks to give a satisfactory fluid milk. Increased milk concentration, higher storage temperatures, or longer periods of storage tended to produce a gelation of the product due apparently to changes in hydration of the casein.

Webb and Hall (10) also found that holding skimmilk in a frozen condition increased the heat stability at  $120^{\circ}\text{C}$ . up to 17 weeks, after which time the heat stability declined. Condensed skimmilk (18% M.S.N.F.) increased in heat stability up to 7 weeks, but subsequently became progressively unstable. They also state that slow freezing of milk or cream caused a gradual precipitation of the caseinate system and an immediate destruction of the fat emulsion.

Doan and Baldwin (11) state that, "Freezing *per se* has no measurable effect on the protein dispersion. Holding in the frozen condition for several weeks or months is required definitely to cause aggregation, denaturation or instability of the proteins."

The effect of long periods of storage in the frozen condition ( $-15^{\circ}\text{C}$ .) on the bound water content of skimmilk and condensed skimmilk ( $2\frac{1}{2}$  to 1) was studied. Whole milk and cream were also frozen with the intention of determining bound water content but due to destabilization of the butterfat on thawing, accurate solids determinations could not be made and this part of the experiment was therefore discarded.

The samples of skimmilk and condensed skimmilk were frozen and stored at  $-10$  to  $-15^{\circ}\text{F}$ . in quart cardboard containers and thawed at room temperature. The skimmilk had been pasteurized at  $143^{\circ}\text{F}$ . for 30 minutes previous to freezing. The results of these experiments are given in Table 10.

The results indicate that holding skimmilk relatively long periods of time in the frozen condition has no great effect upon the alcohol stability of the proteins, but with the condensed skimmilk destabilization occurred. The bound water content of the skimmilk increased slightly and at the 60 day determination it reached its maximum and remained the same up to the 85th day determination. A slight decrease in bound water occurred when determined on the 147th day.

TABLE 10  
*The effect of freezing milk plasma on the bound water content*

Sample	Days frozen	Per cent solids	pH	Viscosity centipoises	Per cent acid	Alcohol no.	Per cent bound water
1. Skimmilk . . . . .	0	9.06	6.55	1.775	0.195	8.5	2.13
	30	9.02	6.45	1.837	0.175	8.5	2.24
	60	9.05	6.43	1.877	0.175	8.5	2.36
	85	9.05	6.43	1.877	0.175	8.5	2.36
	117	9.06	6.48	1.735	0.175	8.3	2.13
	147	9.06	6.49	1.694	0.170	8.2	2.01
2. Condensed Skimmilk	0	25.33	6.15	7.650	0.58	5.4	11.62
	25	25.36	6.05	7.750	0.58	5.5	12.17
	57*	.	.	.	.	.	....

\* Partial precipitation of the casein on thawing.

The condensed skimmilk increased in bound water when stored in the frozen condition for 25 days. After 57 days a marked precipitation of the proteins occurred. Although bound water determinations could not be made, a decrease in the bound water content probably took place. These results parallel the work of Webb and Hall (10), previously cited, on the heat stability of frozen skimmilk and frozen condensed skimmilk.

*The Effect of Added Salts on the Bound Water Content of Concentrated Milk Plasma and of Cream*

According to the Sommer and Hart theory of Salt Balance (12), citrates and phosphates usually increase heat stability of milk proteins while calcium and magnesium are usually detrimental in their effect on stability. Since stability is usually associated with the proteins and salt balance, it is reasonable to believe that these stabilizing and destabilizing salts should produce some effect upon the bound water content of dairy products, especially in ice cream mix, evaporated milk and coffee cream.

To the writers' knowledge very little work has been done with the effect of added salts on the hydration of the proteins. The effect of hydration is relatively important in ice cream mixes. If the salt balance is not proper in an ice cream mix there is a tendency for the proteins to precipitate upon homogenization and from the standpoint of coffee cream there is a tendency for the cream to "feather" in the coffee. The use of salts is prevalent in the evaporated milk industry. The proteins of evaporated milk have a tendency to precipitate on sterilization if the salts are not in proper balance.

Sommer (13) shows that proteins can be precipitated by "salting out" and that this process is known to exert a dehydrating effect. He further states that citrates and phosphates have a hydrating effect on casein while calcium decreases its hydration.

Sommer and Young (14) reported that upon adding 0.40 and 0.56 per cent of sodium citrate or 1.26 per cent di-sodium phosphate the ease of whip-

ping ice cream mixes was greatly increased, while 0.1 and 0.2 per cent calcium lactate had a noticeable effect and 0.5 per cent had a marked effect in reducing the ease of whipping of ice cream mixes. Later Sommer (15) found that these results could not always be duplicated.

Hening and Dahlberg (16) added sodium citrate, potassium oxalate (poisonous), and di-sodium phosphate to ice cream before pasteurization, and homogenization and obtained a lower viscosity and a greater whipping ability. Calcium lactate increased viscosity and fat clumping and made the mix more difficult to whip.

Keith, Rink and Weaver (17) found that the citrate ion greatly decreased the viscosity and fat clumping, and decreased the titratable acidity only very slightly. The stability of the proteins was increased particularly toward precipitation by alcohol. The calcium ion gave exactly opposite results from those of the citrate ion.

The effect of sodium citrate and di-sodium phosphate on the bound water content was determined with concentrated milk plasma. The sample was divided into three parts as follows: (1) control; (2) control plus one per cent N/4 sodium citrate; and (3) control plus one per cent N/4 di-sodium phosphate. These samples were pasteurized at 143° F. for 30 minutes, cooled to 40° F. and determinations made, after aging at 40° F., at 4 and 24 hours. The results are given in Table 11.

TABLE 11

*The effect of milk stabilizing salts on the bound water content of concentrated milk plasma*

Sample	Hours aged at 40° F.	Per cent solids	pH	Viscosity centipoises	Per cent acid	Alcohol no.	Per cent bound water	Grams bound water per gram solids
1. Control	4	11.87	6.39	2.959	0.24	8.2	4.31	0.363
	24	11.87	6.41	3.020	0.24	8.3	4.64	0.391
2. Sod. Citrate	4	11.81	6.45	3.061	0.225	8.7	4.83	0.409
	24	11.81	6.46	3.122	0.225	8.8	4.95	0.419
3. Di-sodium Phosphate	4	11.83	6.46	3.020	0.225	8.7	4.95	0.418
	24	11.83	6.49	3.102	0.22	8.9	5.42	0.458

The results in Table 11 show that sodium citrate and di-sodium phosphate increase the hydration of the proteins as indicated by a slight increase in bound water content. These stabilizing salts also caused an increase in protein stability. The effects produced on the pH and the acidity do not appear significant although there was a slight reduction in acidity.

The same experiments were repeated using cream instead of milk plasma to note the effects of these stabilizing salts on the bound water content. Table 12 shows the results obtained with these stabilizing salts in cream.

TABLE 12

*The effect of milk stabilizing salts on the bound water content of 25 per cent cream*

Sample	Hours aged at 40° F.	Per cent solids	pH	Viscosity centipoises	Per cent acid	Alcohol no.	Per cent bound water	Grams bound water per gram solids
1. Control .....	4	31.44	6.61	8.641	0.13	7.0	5.90	0.188
	24	31.44	6.63	8.741	0.135	7.1	6.18	0.196
2. Sod. Citrate	4	31.16	6.66	7.857	0.12	7.0	6.01	0.193
	24	31.16	6.71	9.387	0.123	7.15	6.43	0.206
3. Di-sodium Phosphate .....	4	31.24	6.63	7.714	0.125	7.0	6.04	0.190
	24	31.24	6.68	8.877	0.127	7.2	6.43	0.205

The effect of these stabilizing salts on the bound water content of cream is not marked. The results indicate that these salts do increase the bound water content slightly. It appears, therefore, that the effect produced by sodium citrate and di-sodium phosphate is mainly on the substances in the plasma and not on the substances surrounding the fat globules.

The salts antagonistic in their action upon protein stability are the salts of calcium and magnesium. As a rule they are known to have a destabilizing effect upon the milk proteins. The salts used in this study were calcium phosphate (monobasic) and calcium lactate. These salts were added also on a normality basis to concentrated milk plasma which was then pasteurized at 143° F. for thirty minutes after which it was cooled to 40° F. and determinations made after aging 4 and 24 hours.

TABLE 13

*The effect of destabilizing salts on the bound water content of concentrated milk plasma*

Sample	Hours aged at 40° F.	Per cent solids	pH	Viscosity centipoises	Per cent acid	Alcohol no.	Per cent bound water	Grams bound water per gram solids
1. Control .....	4	14.26	6.32	3.102	0.310	7.7	7.49	0.525
	24	14.26	6.29	3.449	0.310	7.8	7.73	0.542
2. Monocalcium Phosphate	4	14.14	6.26	3.020	0.330	6.8	6.96	0.492
	24	14.14	6.26	3.245	0.330	6.85	7.07	0.500
3. Calcium Lactate .....	4	14.22	6.29	3.020	0.310	6.8	7.03	0.494
	24	14.22	6.31	3.265	0.313	6.9	7.17	0.504

The hydration of the proteins as measured by the amount of bound water present shows that the so-called destabilizing salts are only slightly detrimental to the bound water content and the stability of the proteins. Both calcium phosphate and calcium lactate decrease the amount of bound water about the same degree. There was also a considerable decrease in protein stability.

The effect of these destabilizing salts on cream was also studied. The

salts were added in the same amounts as previously described and the cream was also pasteurized at 143° F. for thirty minutes.

TABLE 14

*The effect of destabilizing salts on the bound water content of 27 per cent cream*

Sample	Hours aged at 40° F.	Per cent solids	pH	Viscosity centipoises	Per cent acid	Alcohol no.	Per cent bound water	Grams bound water per gram solids
1. Control	4	33.61	6.51	13.510	0.155	6.0	5.57	0.166
	24	33.61	6.54	16.531	0.160	6.2	5.82	0.173
2. Monocalcium Phosphate	4	33.27	6.44	13.061	0.170	4.7	4.99	0.149
	24	33.27	6.44	14.898	0.170	4.8	5.13	0.154
3. Calcium Lactate	4	33.25	6.58	12.595	0.165	4.5	4.51	0.136
	24	33.25	6.58	15.000	0.170	4.6	4.62	0.138

The effect of destabilizing salts on the bound water content of cream is noted above. Both salts studied appear to decrease the amount of bound water. This decrease in bound water is accompanied by a decrease in viscosity and a decrease in protein stability.

#### CONCLUSIONS

The bound water content of a prepared casein sol is greatest at a pH of approximately 6.6–6.7 and tends to diminish if the pH is either increased or decreased. Concentrated milk plasma has the greatest amount of water bound at a pH 6.2–6.4 and tends to diminish if the pH is either increased or decreased.

The bound water content per unit of casein tends to diminish with the concentration of the casein sol. Raising the heating temperature lowered the bound water content of the concentrated milk plasma. High pasteurization temperatures decrease the bound water content of the fat globule “membrane” and of pure milk phospholipids.

Pasteurization of cream at 143° F. for 30 minutes decreases only slightly the bound water content while homogenization decreases the bound water content still further. Homogenization decreases the bound water content of “ice cream mixes.” The higher the pressure of homogenization the greater was the reduction of bound water content. Destruction of the clumps by dual homogenization increases the stability of the proteins and increases the amount of bound water.

The freezing of skimmilk and condensed skimmilk over long periods of time reduces the protein stability and the bound water content.

The so-called milk stabilizing salts tend to increase slightly the bound water content and protein stability of concentrated milk plasma and cream,

while the destabilizing salts tend to decrease the bound water content and protein stability of concentrated milk plasma and cream.

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## PEDIGREE PROMISE AND PROGENY TEST AMONG SIRES PROVED IN IOWA COW TESTING ASSOCIATIONS<sup>1</sup>

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Bulls which were proved in Iowa Cow Testing Associations, according to the rules of the Dairy Bureau in effect before 1936, were studied to see (1) how many of their parents and grandparents had been tested for production and (2) how closely the production testing in the bull's pedigree indicated either the average production of his daughters or the average increase of his daughters over their dams. This study was confined to the 303 Holstein-Friesian sires (which constituted about half of all sires proved) so that differences in breed averages would not obscure the intra-breed situation, which alone is of practical interest to the breeder who follows a policy of pure breeding or of consistently grading toward one pure breed. We see no *a priori* reason to suppose that the relation between pedigree promise and progeny performance would be different in other breeds.

Each bull had at least five tested daughters out of cows also tested in C.T.A. herds. About half of the bulls had *only* five such daughter-dam pairs. Less than one-fifth of them had more than eight pairs. For the present study the average fat production of the daughters (or of the mates<sup>2</sup>) of each bull was treated as a single item, regardless of the number of daughters (or mates) in that average. About half of the bulls were proved by association-year records; that is, by using as the measure of the cow's production her total production during the twelve months of the association year, regardless of her stage of lactation or lactations. The others were proved by lactation records; that is, by using for the cow's production her production during her whole lactation, or during the first 365 days if her lactation was longer than that. The association-year records were corrected to maturity by considering the two-year-old record as 70 per cent, the three-year-old record as 80 per cent, and the four-year-old record as 90 per cent of the mature record. The lactation records were corrected to maturity by the Bureau of Dairy Industry

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<sup>2</sup> The dams of the bull's daughters are hereafter called the bull's mates, to distinguish them from the bull's own dam.

factors now in general use. When a cow had more than one record the highest was used. No case was included where the daughters or mates were known to have been milked more than twice a day but, as the number of times milked was not noted on the earlier reports used in proving sires, a few such cases may be included.<sup>3</sup>

As production records for the bulls' parents and grandparents we used only Advanced Registry records more than 305 days in length.<sup>4</sup> All such records completed before the end of 1936 were included. These records were corrected to the "mature B" basis (mature cows milked three times a day) by the factors used by the Holstein-Friesian association. Most of the tested dams and grandams of course had only one A.R. record. Where a cow had more than one A.R. record the average of those was used. For each sire and grandsire the average of the A.R. records of all his daughters was used as his A.R. test.

When the daughter average, the mate average and the average increase of each sire's daughters over his mates were each treated as single items, the means and standard deviations<sup>5</sup> of those items for these 303 sires were as follows:

	Pounds of butterfat	
	Mean	Standard deviation
Mates . . . . .	416	78
Daughters . . . . .	430	82
Increase . . . . .	14	68

<sup>3</sup> Correlations between individual daughters and dams within the group used to prove each sire indicate that individual differences in the fat records were about one-fourth caused by hereditary differences between the cows. Differences in test (percentage of fat in the milk) were about one-half caused by hereditary differences between the cows. The lactation records and the association-year records seemed about equal in their accuracy as indicators of the cow's heredity (*JOURNAL OF DAIRY SCIENCE* 19: 429-430). A later study of 305-day lactation records used since 1935 in proving bulls in Iowa indicated that intra-herd differences in unselected single records of fat production were about 43 per cent permanent throughout the cow's lifetime and about 28 per cent hereditary (*JOURNAL OF DAIRY SCIENCE* 20: 440-441). We hope that these studies of heritability can be extended soon.

<sup>4</sup> Many of the dams and some of the grandams of these bulls had C.T.A. records, but those were not at that time systematically recorded in any one place where we could inquire what records existed concerning each cow or bull. We can only guess at the amount of C.T.A. testing in the pedigrees of these bulls. Among 1,055 bulls in use in Iowa C.T.A. herds in 1937, 45 per cent were out of C.T.A. tested dams and another 39 per cent were out of A.R. tested dams with 4 per cent being out of H.I.R. tested dams and only 12 per cent being from dams not tested at all. Since the 303 bulls studied here were from an earlier time (already proved before the end of 1935), it seems certain that the proportion of C.T.A. testing in their pedigrees was less than this, but we do not know how much less.

<sup>5</sup> These standard deviations and the correlations were corrected for what little heterogeneity there was between lactation and association-year records.

The correlations between these three items were as follows :

Mates and daughters .....	+ .64
Mates and increase .....	-.38
Daughters and increase .....	+ .47

The rather high correlation between the average of the bull's mates and the average of his daughters comes from two biologically diverse sources. The first is the genetic fact that daughters get half of their inheritance from their dams and hence tend to resemble those dams somewhat—a correlation which the process of averaging makes fairly high between *averages* of a bull's mates and *averages* of his daughters, even when it is low between *individual dam* and *individual daughter*. The second is the heterogeneity of management and feeding practices from one herd to another, whereby dam and daughter (which are nearly always tested in the same herd) would in many cases have had their records both raised or both lowered by the environmental circumstances characteristic of that herd but not of all herds. The negative correlation between mates and increase is only another way of expressing the well-known general tendency for offspring to regress from a parent toward the mean of the race, this regression itself resulting from two quite distinct causes. The first of these is that the size of a cow's record is affected by other things besides her breeding value and therefore the cows with the highest records generally do not have as good heredity as their records (if taken at face value) would indicate, while the cows with the lowest records generally are not quite as poor as their records. The other fact causing this regression is that the best cows are not always mated to the best bulls and the poorest cows are not always mated to the poorest bulls. The daughters, getting half of their inheritance from each parent, therefore generally tend to be poorer than their dams in the case of those with the very best dams and better than their dams in the case of those with the poorest dams. The positive correlation between the daughters and the increase is another way of expressing that same regression.

That the 14 pound average increase in one generation measures the true rate of genetic improvement in this population can hardly be maintained with confidence. Too many other possibilities may have affected this figure. For proving the sire only the best record was used when a cow had more than one, and many of the daughters had only made one record when their sire was "proved." Hence the records of the dams in general probably were a bit more highly selected than the records of the daughters. There seems to be a general tendency for herds to improve their management with increasing experience in C.T.A. testing. In many cases the daughters made their records at a later date than their dams and would have been under better management. It is possible that greater effort was made to gather the "proof" on sires which were thought to be doing well than on those which were believed

to have been poor sires, even though the testers were urged to prove all bulls without regard to the level of that proof. These considerations lead us to think that this 14 pounds average increase is of little use as a guide to the rate of genetic change in this population.

The averages of the lactation-year records and of the association-year records, in pounds of butterfat, were as follows:

	C.T.A. year	Lactation year
Number of bulls .....	149	154
Mates' production .....	403	429 -
Daughters' production .....	431	429 +
Increase .....	28	1 -

There was very little difference in the production of the daughters. The C.T.A. year records mostly come from a slightly earlier period than the lactation records, although there was some overlapping in those dates.

#### DESCRIPTION OF PEDIGREES

Table 1 shows, as a composite pedigree, the averages and variabilities of the A.R. tests of parents and grandparents. About half of the sires, nearly

TABLE 1

*Composite pedigrees showing the amount of A.R. testing*

303 Sires Proved in Iowa C.T.A.	Sires: 161 had A.R. daughters averaging 631 lbs. $\sigma = 76$ lbs. Quartiles: 580 and 682	Paternal Grandires: 223 had A.R. daughters averaging 636 lbs. $\sigma = 63$ lbs. Quartiles: 594 and 678
	Dams: 62 had A.R. records averaging 645 lbs. $\sigma = 105$ lbs. Quartiles: 574 and 716	Paternal Grandams: 55 had A.R. records averaging 731 lbs. $\sigma = 109$ lbs. Quartiles: 657 and 805
		Maternal Grandires: 116 had A.R. daughters averaging 619 lbs. $\sigma = 71$ lbs. Quartiles: 571 and 667
		Maternal Grandams: 14 had A.R. records averaging 595 lbs.

three-fourths of the paternal grandsires, and more than a third of the maternal grandsires had A.R. daughters.<sup>6</sup> The averages for sires and for the two grandsires were nearly the same and the differences which did appear were statistically insignificant, although in the direction to be expected if such records actually do carry some weight in determining the buyer's choice of bulls.

The standard deviations of these sire tests are about the same for sires and both grandsires. Quartile values (computed from these standard deviations) are shown for convenient use in estimating at a glance whether a particular sire's daughters are extremely high-producing or only moderately so, as compared with daughters of other sires. These quartile values indicate closely enough for practical purposes the boundary between the lowest quarter and the next to the lowest quarter and the boundary between the highest quarter and the next to the highest quarter of the bulls.

The records of the dams and grandams indicate that the most intense selection in dairy cattle breeding is practiced in deciding which dams are good enough to have their sons saved (the high average of the dam) or to have their sons stand at the head of purebred herds (the very high average of the paternal grandams). The general average of all mature A.R. records in Class B in the Blue Book to the end of 1936 is 619 pounds of fat. Differences between this figure and the averages shown in Table 1 may perhaps indicate how slight after all is the selection actually practiced in favor of high production when bulls are chosen to head even such progressive C.T.A. herds as the ones in which these sires were proven.

The records of dams and grandams show larger standard deviations than the records of sires and grandsires. In most cases the sire's and grandsire's records are averages of several daughters, whereas many of the dams and grandams had but one record and few had as many as three. Averages are naturally less variable than single records. The lower variability of the records of male ancestors probably results automatically from the averaging process and does not indicate at all that the male ancestors vary less than the female ancestors in their breeding values. An additional indication of this is the fact that the averages of the bulls' mates in the C.T.A. records are about as variable as the daughter averages for the sires and grandsires.

#### PEDIGREE AS RELATED TO PROOF

For each ancestor in turn, the 303 bulls were grouped in two groups according to whether that ancestor was or was not A.R. tested. Table 2 shows the average production of mates and of daughters for each of these groupings. The most conspicuous feature of Table 2 is that the bulls with testing in their pedigrees were mated to cows of higher production than were

<sup>6</sup> It should be remembered that some of the others had C.T.A.-tested daughters, but the records of these were not assembled so that we could consult them.

TABLE 2

*Differences in the performance of 303 sires according to whether they had or did not have A.R. testing in various parts of their pedigrees*

Part of the pedigree concerned:		Averages pertaining to the proof of bulls whose ancestor was:		Difference
		Tested	Not tested	
Sire tested or not tested:	Number of bulls .....	161	142	
	Mates of these bulls .....	427	403	+ 24
	Daughters of these bulls ..	441	417	+ 24
	Increase .....	+ 14	+ 14	+ trace
Dams tested or not tested:	Number of bulls .....	62	241	
	Mates of these bulls .....	444	409	+ 35
	Daughters of these bulls ..	444	427	+ 17
	Increase .....	- trace	+ 18	- 18
Paternal grand-sire tested or not tested:	Number of bulls .....	223	80	
	Mates of these bulls .....	421	401	+ 20
	Daughters of these bulls ..	435	417	+ 18
	Increase .....	+ 14	+ 16	- 2
Paternal grandam tested or not tested:	Number of bulls .....	55	248	
	Mates of these bulls .....	430	413	+ 17
	Daughters of these bulls ...	428	431	- 3
	Increase .....	- 2	+ 18	- 20
Maternal grand-sire tested or not tested:	Number of bulls .....	116	187	
	Mates of these bulls .....	431	406	+ 25
	Daughters of these bulls ...	435	427	+ 8
	Increase .....	+ 4	+ 21	- 17
Maternal grandam tested or not tested:	Number of bulls .....	14	289	
	Mates of these bulls .....	437	415	+ 22
	Daughters of these bulls ...	458	429	+ 29
	Increase .....	+ 21	+ 14	+ 7

the other bulls. For all six ancestors, except the paternal grandam, those bulls with tested ancestors had daughters averaging higher than the daughters of the bulls from untested ancestors. Because both the mates and the daughters were higher producers where there was testing in the pedigree, the increase of daughters over dams was for four of the six ancestors less for the bulls which had testing in their pedigrees than for bulls without such testing. We interpret Table 2 as indicating that the men who have the higher producing cows pay more attention to selecting their bulls than do the men who have the lower producing cows. By this greater effort they maintain their production at a higher level but they do not make it rise toward still higher levels any faster than it does in the herds where the bulls do not have A.R. testing in their pedigrees. Doubtless there is considerable regression toward

the breed average, both downward from those which have unusually good pedigrees and upwards from those whose pedigrees seem very poor.

Since these sires were proven before the end of 1935, even the youngest of them must have been placed in service before the end of 1931. Many of them were initially selected years earlier than that. On the other hand in some cases the A.R. records in their pedigrees were not made until near 1936. Therefore the comparisons as made here between those with A.R. testing in their pedigrees and those without such testing, include information which the purchaser of the bull in many cases could not have known when he selected that bull.

Considering one ancestor at a time and taking all cases in which that ancestor was A.R. tested, we measured the correlation between the bull's performance and the A.R. test of the ancestor and found the facts shown in Table 3.<sup>7</sup> The question here is how closely the size of the A.R. record indicates a correspondingly good or poor performance of the bull in siring daugh-

TABLE 3

*Correlations between the bull's daughters' production (average as in columns 4 and 5, or increase over dams as in column 6) and the A.R. tests of his ancestors*

Ancestor whose test was correlated with the bull's performance	Number of bulls included	Approximate standard error of r	Bull's performance		
			Average of his daughters		Increase of his daughters over their dams
			Regardless of their dams	From cows of a given level of production	
Sire . . .	161	.08	-.02	-.02	-.01
Dam . . .	62	.13	+.24	+.11	-.06
Paternal grandsire	223	.07	+.06	+.10	+.11
Paternal grandam	55	.14	+.10	+.21	+.23
Maternal grandsire	116	.09	+.03	+.01	-.01

ters which average high in production or which produce more than their dams. None of the correlations in Table 3 are statistically significant. They are prevailingly positive, but small. The partial correlations between the performance of the bull's ancestor and the performance of the bull's daughters from cows of a given level of production, we think are a bit more dependable than the simple correlation coefficients as indicators of the relation between ancestor's record and bull's performance. The partial correlations are less likely to be distorted by possible relations between the level of testing in the bull's pedigree and the level of production of his mates.

<sup>7</sup> Corrected for the slight heterogeneity between lactation and association-year records.

We interpret Table 3 as indicating that the relation between the bull's pedigree and his performance is positive but slight. But in making this interpretation it must be remembered that here the ancestors are considered one at a time, whereas the purchaser of a bull may combine the high records of some ancestors and the low records of others in the same pedigree to arrive at some kind of a weighted estimate of the desirability of the pedigree as a whole. The correlation between such a weighted estimate of the pedigree and the bull's performance would usually be larger, although not vastly so, than the correlations between any one of the ancestors and that performance. We were not able to combine the record of sire and dam and grandparents into a single multiple correlation prediction equation for the bull's own performance, because so few bulls in these data had A.R. tests for all six of those ancestors. A second qualification bearing on this interpretation is that we are here correlating the pedigree record with the *observed* progeny performance. That the progeny test of the bull is not a perfect indication of his breeding value is evident from the fact that the progeny test based on his first five or first ten daughters may be different from a similar test based on his subsequent daughters. That is, in comparing a pedigree with the observed progeny test we are correlating one indication of the bull's breeding value with another indication of the same thing, neither indication being perfectly reliable although one may be more so than the other. Under such circumstances the correlation between either of those indications and the bull's true breeding value, if the latter could be measured accurately, would almost certainly be somewhat higher than the correlation of the one indication with the other. Taking all these things into consideration, we think these correlations give slightly too low an estimate of the real usefulness of the A.R. records in a bull's pedigree as indicating his breeding value.

#### DISCUSSION

In nearly half of our data the progeny tests of the bulls themselves include only five daughters. Only an eighth of our bulls were proved by as many as ten pairs of daughters and dams. The figures for sires and grandsires in our study included all the tested daughters of each, but in many cases this was as few as three and in but few cases did it go far above ten.

Our correlations are lower than those found by Copeland<sup>a</sup> or by Madsen<sup>b</sup> in the only other reports we have seen of studies closely similar to this one. They found correlations as follows between the average production of a bull's daughters (the production of the bull's mates not being considered) and the production records of the bull's near ancestors:

<sup>a</sup> Copeland, Lynn. 1934. Pedigree Analysis as a Basis of Selecting Bull Calves. *JOUR. DAIRY SCIENCE* 17: 93-102.

<sup>b</sup> Madsen, Karl. 1932. Inheritance of Milking Capacity. *Nature*, January 30, 1932.

	Copeland	Madsen	
	Lbs. fat	Lbs. fat	Lbs. milk
Sire's daughters . . . . .	+ .56	+ .32	+ .25
Dam's own records . . . . .	+ .33	+ .18	+ .17
Paternal grandsire's daughters . . . . .	+ .25	+ .19	+ .20
Maternal grandsire's daughters . . . . .	+ .43	+ .26	+ .19
Paternal grandam's own records . . . . .	..	+ .06	+ .03
Maternal grandam's own records . . . . .	..	+ .17	+ .11

There are several differences in the data studied. Perhaps the chief one is that Copeland and Madsen included only data on bulls which had at least ten tested daughters. In Madsen's data the average number of daughters per bull was 18 and the average number of records per cow among the dams and grandams was 5.5.

The larger numbers available for the studies of Copeland and Madsen should have tended to give their figures greater dependability and to make them fluctuate less but we cannot see that our figures would have been biased toward lowness by this reason. They might as well have been too large—the scantiness of the material would merely permit them to be more erratic. Our material corresponds closely to the situation which the bull purchaser of today must face. The figures we studied were as much information<sup>10</sup> as the prospective purchaser could get about this bull in early 1937.

It may be worth while to point out that these correlations should be compared not with perfect correlations (which are not to be expected in any case) but with what might be expected in the limiting case in which the differences between cows as revealed by their records or between bulls as revealed by their daughters' records are assumed to be *perfectly* hereditary. This depends in part upon the degree of assortive mating practiced among those animals which do get selected to be parents but, as all dairy breeders are striving for high production, only the fact that some breeders strive harder or more wisely than their fellows toward the common goal can give rise to assortive mating. This surely cannot be intense in the population as a whole. Not enough inbreeding or extreme outbreeding is practiced that this would alter the picture noticeably. Ignoring for the moment the traces of assortive mating and inbreeding which probably exist, the expected correlation between the record of the bull's dam and the average record of his daughters would rise from + .25 toward + .50 as the number of his daughters increased. Similarly the expected correlation between the average record of the bull's sire's daughters and the average record of the bull's own

<sup>10</sup> Except that we did not use 7-day and 30-day records which were more abundant in the older pedigrees, H.I.R. records which are beginning to be available in the pedigrees of young bulls, and C.T.A. records which until recently have not been assembled systematically at any one place and have mostly been left in the barn books of the C.T.A. member in whose herd they were made.

daughters would range from  $+ .125$  when each had but a single daughter toward  $+ .50$  if each had very many daughters. The correlations between the bull's daughters and his grandparents' records would be just half as large. If all of the bull's ancestors were thoroughly tested and if their records could all be considered at once, the expected correlation between the promise of the pedigree as a whole and the bull's actual performance would range up toward  $+ .71$ , being restrained by the sampling nature of Mendelian inheritance from going higher than that.<sup>11</sup> The existence of some assortive mating causes the correlation expected when one ancestor is considered at a time to be somewhat higher but has little effect on the dependability of the whole pedigree. The assortive mating merely results in each ancestor indicating to some extent what the other ancestors will be. Therefore the assortive mating causes one ancestor by itself to be a more useful indicator than it would be under random mating but not so much additional information is gained by actually learning the facts about those other ancestors. Perhaps little is to be gained by discussing these abstract conditions further, since in actual practice we face the overwhelmingly important fact that the size of a cow's record or of a bull's daughter average, even under A. R. conditions, is much influenced by many things other than the cow's or bull's heredity and that many of these things are dependent on the herd management or environment in such a way that no amount of increase in the number of records or number of daughters tested will tend to equalize those environmental differences as between animals which are tested in different herds.

That a few of Copeland's and Madsen's correlations are higher than expected, even on the assumption of perfect heritability, might result from undiscounted time trends or from stratification of management practices whereby there may be a noticeable tendency for a buyer to get his bull from a breeder who manages and tests his herd according to somewhat the same standards as the buyer does. Or if the divergence of ideals between those who put type first and those who put production first is distinct (as differences in emphasis could make it, even when all breeders are paying some attention to both type and production) and if there are many holding each ideal, then the assortive mating thus introduced could raise the correlations with individual ancestors above the limits expected under the abstract conditions. It seems profitless to speculate farther in this direction now, but we do think it worth while to mention that some of Copeland's and Madsen's correlations are rather higher than the general evidence about heritability

<sup>11</sup> This is the multiple correlation to be expected between the genotype of an individual offspring and the genotypes of its two parents in a population mating at random. No amount of knowledge about the ancestors can lead farther than toward a perfect knowledge of the genotypes of the two parents. See Genetics 6: 111-161 for more details concerning these biometrical relations.

of differences in milk and fat production leads us to anticipate, whereas our own coefficients are somewhat lower than we had anticipated.

The practical implications of these studies are that pedigree promise is worth something in selecting a bull but as a cold-blooded business proposition it should not be valued too highly. As a usual thing the prospective purchaser can reasonably expect to get in the daughters of his bull only a small part of that superiority over the rest of the breed which his bull's parents and grandparents showed. Often the pedigree is almost the only guide which the purchaser has, besides seeing that the bull meets his minimum standards of type and health. Like the weather forecasts, pedigree promise has some value, even if it isn't infallible! But, just as the traveler on a day when fair weather is predicted may take along an umbrella or raincoat if it doesn't cause too much expense or trouble, so the purchaser of a bull with even an extraordinarily good pedigree may find it best to sample the bull fairly and wait to learn the results before he builds his breeding plan too extensively around that bull, if the cost of sampling and waiting isn't too large. The more one knows about the conditions under which the records in the pedigree were made and allows for the differences which those conditions made in the records, the more accurate the pedigree becomes as an indicator of the bull's breeding value, but this is sharply limited both by the fact that one cannot make absolutely perfect allowances for those conditions, no matter how well he knows them, and also by the biological limits which the sampling nature of inheritance places on the accuracy of pedigree estimates. The existence of these distinct limits makes the law of diminishing returns apply to the effort spent in detailed study or standardizing of pedigrees. It is often worth while to find out the main features about the conditions under which the bull's ancestors were tested—*e.g.*, whether their records are selected ones or life-time averages, what the average production of the other cows in the herd was in the same years, whether they were milked oftener than twice a day, and roughly how well they were fed—but one quickly comes near the point where further study of these details will improve the accuracy of the pedigree estimate so little that such further study is scarcely worth the trouble.

#### SUMMARY

A study of the pedigrees of 303 Holstein-Friesian bulls proved in Iowa Cow Testing Associations before 1936 showed:

1. That the bulls with A.R. testing in their pedigrees were used in higher producing herds and had higher producing daughters than the bulls without such testing but, because of the higher production of their mates, the bulls with testing in their pedigrees did not increase the production of their daughters any more than the others did.

2. The correlations between ancestor's A.R. records and the bull's progeny performance were prevailingly positive but were so small as to be statistically insignificant on the amount of data available.

Although so limited in amount as to make them subject to large sampling errors, the data are compatible with the general conclusion that it is desirable to select bulls which have tested ancestors and whose tested ancestors have high records but that one can expect to gain in the daughters of such bulls only a small fraction of that superiority which their tested ancestors showed, as compared with the breed average.

## OBSERVATIONS ON THE SPLITTING OF BRICK CHEESE

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At certain seasons of the year, a very characteristic defect may appear in Brick cheese when it is seven to fourteen days old. Excessive development of gas in the cheese causes holes which commonly become large enough to split the cheese. This defect is sometimes called "late-gas" in order to distinguish it from the gas which occasionally forms during the making or draining processes. The defect may appear in one part of a cheese as shown in figure 1, or it may extend throughout the length of the cheese, as

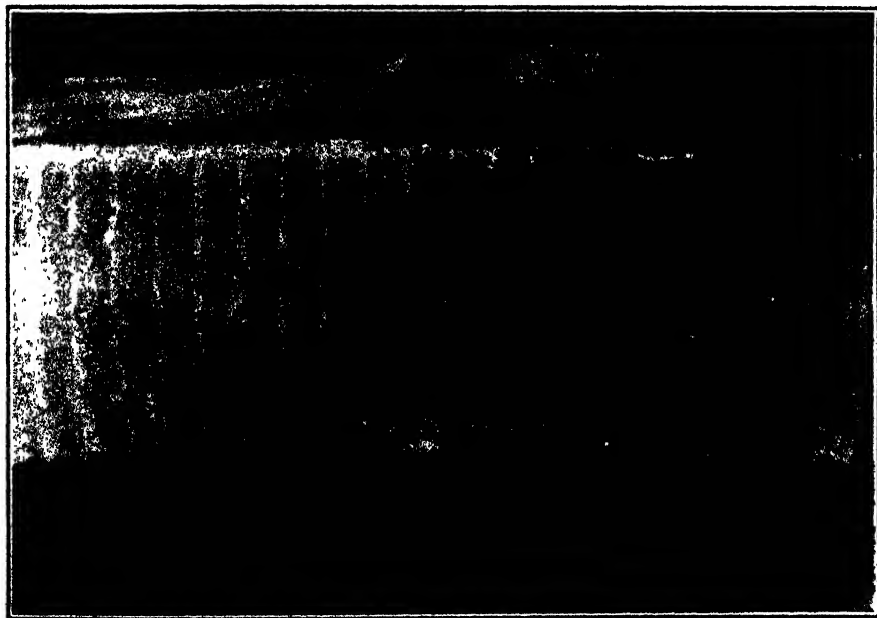


FIG. 1. Swelling of the cheese in a small area may occur when the gas development is localized. (The cheese is about 3 in. high at the edge. The whole cheese is not shown in this picture.)

illustrated in figure 2. A typical cross section of a defective cheese is shown in figure 3. This figure illustrates not only the splitting but also the "sweet" holes, which are sometimes associated with the splitting. Frequently a flat, metallic flavor appears in the cheese, even before the swelling occurs; at other times the cheese has a rather pleasing, sweet flavor. The defect has been observed most frequently in Winter and early Spring.

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FIG. 2. Swelling of the cheese may take place throughout the entire length of the cheese when gas development is general. (The length of the cheese is 10 in.)

It occurs in some factories year after year, sometimes affecting all and sometimes only a portion of each day's make. Such cheese cannot be readily sold on the retail market.

It has been possible to observe the defect in this laboratory and in com-

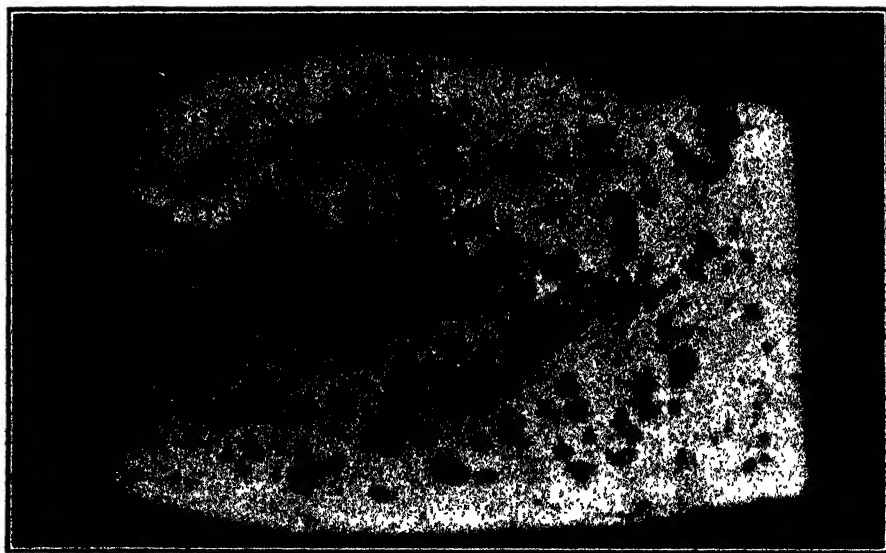


FIG. 3. A cross section of a cheese showing the splitting defect and numerous gas holes. (The size of the cheese section is approximately  $3 \times 5$  in.)

mercial plants from time to time during the past six years. It is the purpose of this discussion to point out some of the conditions which seem to be associated with its occurrence in the cheese.

When the defect was first observed in our manufacturing laboratory, special precautions were taken to sterilize all cheese equipment and to change the starters used in the manufacture of the cheese. Milk for cheese making was pasteurized and every precaution was taken to avoid subsequent contamination. Despite these precautions, the cheese continued to develop the characteristic splitting. Process cheese made from this defective cheese also developed a similar type of fermentation. Undoubtedly the milk from which the cheese was made contained the responsible organisms when it was delivered by the farmers, and ordinary pasteurizing temperatures failed to destroy them.

#### EXPERIMENTS FROM 1933 TO 1935

It seemed probable that the gas-producing organisms were spore-forming anaerobes. Samples of the gassy cheese were, therefore, ground and placed in flasks containing sterile milk, to which a small amount of reduced iron had been added. These flasks were incubated at 37° C. for 15 hours. Such violent fermentations occurred in several of the flasks that the cotton plugs were dislodged. A number of pure cultures of spore-forming anaerobes were eventually obtained from these sources. Mixtures of several of the more active of these cultures were used to inoculate milk for cheese when it was considered necessary to obtain the defect. The futility of such inoculations was discovered eventually when controls as well as inoculated lots developed the defect.

From 1933 to 1935 several conditions of manufacture were studied, including: variations in the ripening of milk for cheese making; the type of starter; temperature of heating; time of dipping; moisture content of the cheese; methods of salting; the amount of salt in the cheese and the effects of pasteurization. These experiments will not be discussed in detail, but some of the more interesting relationships will be indicated.

Varying the time of ripening the milk before setting was carried to an extreme by holding the milk, to which normal amounts of *S. lactis* starter had been added, for two hours at the setting temperature before adding the rennet. The unripened milk was mixed with the necessary amount of rennet immediately after the starter was added. The ripened milk usually developed maximum or excessive amounts of acid, but the cheese was closer in texture, as might be expected, than that made from the unripened milk. Several lots of cheese from both ripened and unripened milk, however, were criticized for gas development and splitting.

*S. lactis*, *L. bulgaricus*, and *S. thermophilus* cultures were used in paired experiments in 1933. Each starter culture was used alone in pasteurized

milk, and utmost care was taken in the manufacturing process to prevent transfer of organisms from one vat to another.

The results indicated that the formation of excessive gas in the cheese after a week or more of curing might result in two distinct types of open-texture defect, depending upon the acidity of the cheese itself rather than upon the acid-producing organism used as starter. Cheese made with *L. bulgaricus* had the greatest and that made with *S. thermophilus* the least acid development. Cheese containing *L. bulgaricus* showed the splitting defect; that made with *S. lactis* showed both splitting and excessive gas formation; while the use of *S. thermophilus* culture tended to develop cheese with many very large holes but with practically no splits. High acid development had a slight suppressing effect upon the formation of gas. Apparently the short-bodied, acid-type cheese tended to split easily following an excessive gas formation which, in sweet cheese with more elastic body, only produced large gas holes. When splitting occurred in sweet cheese, it was preceded by the formation of large holes which finally broke into the characteristic splits when the elastic limit of the cheese was exceeded.

It is usually supposed that high moisture in cheese tends to exaggerate fermentation defects. This does not seem to apply, however, to the splitting defect. Moisture was regulated by changing the time of dipping and by using different temperatures of heating. The results indicated that the differences could be attributed more to the extent of acid developed than to the amount of moisture remaining in the cheese. For example, when the time of dipping was varied, the moisture content of the cheese ranged between 37.7 and 42 per cent. The lowest texture score was given cheese with a moisture content of 38.7, and the best score to cheese with 42 per cent moisture. In general, whenever the conditions of manufacture in these experiments induced the production of a sweeter type of cheese, the judges found more of the characteristic openness than in lots made from identical milk in which more acid was developed during the manufacturing process.

The effects of salt were studied on three different days by dividing the cheese from one vat into four groups. Each group was given a different salting treatment so that the average salt content of the lots of cheese in these four groups averaged 2.0, 2.4, 2.5 and 2.9 per cent. These differences were obtained by exposing the cheese for different periods of time to the brine and by varying the washing treatments by using brine for one group and fresh water for another. All four groups showed some gas defects because the pasteurized milk from which the cheese was made had been inoculated with cultures of anaerobic gas-forming types of organisms. The groups which averaged 2.0 and 2.4 per cent salt were regarded as less desirable in texture by the judges because of the presence of the late-gas and splitting defect in some of the cheese in these groups.

The association in this experiment of the defective texture with the

lower salt groups suggested that the size of the cheese loaves, one of the commonest causes of variations in salt in Brick cheese, might be a significant factor in relation to the defect. Measurements in 1934 and 1935 of texture, salt and thickness of loaves before salting were available on fifty-five lots of cheese made, not only during studies of the gas-defect, but during other experiments as well. Averages of these values are summarized in Table 1. The trends of the average values shown in this table indicate that

TABLE 1

*Relation between texture score and the average thickness of loaves and salt content of Brick cheese*

Texture score*	No. of lots	Thickness	Salt content
		<i>inches</i>	<i>%</i>
Below 2.0	1	1.38	3.93
2.0-2.9	17	2.60	3.16
3.0-3.9	25	2.64	3.29
4.0-4.9	10	2.80	2.96
Above 5.0	2	2.63	3.05

\* 1=excellent; 2=desirable; 3=satisfactory; 4=objectionable; 5=very objectionable.

when the thickness of the cheese increases, there is a loss in texture score. This loss can be attributed to all defects, including "splitting." It is interesting that the lowest average salt contents in this table tend to be associated with the cheese of inferior texture. The thickness of the cheese, however, is apparently more closely related to the texture score than is the salt content. This may be explained by the relation between thickness of cheese and the formation of mechanical and gas holes.

Mechanical holes in Brick cheese are probably first formed by the trapping of free whey between curd particles. Gradually the whey escapes between the curd particles or is absorbed by the curd as it cools. If the whey is released before the curd cools too much, the curd flows into and closes, or partially closes the cavities left by the whey. Since whey escapes more rapidly from small loaves of cheese, such loaves would be expected to have fewer mechanical openings than large loaves.

In a similar manner, it is to be expected that more of the gas formed in small cheese would diffuse through it and escape without forming holes. These combined facts partially explain the relative freedom from either mechanical or gas holes of the layer of curd immediately beneath the rind of the cheese pictured in Figures 1, 2 and 3.

The use of salt to control fermentations is generally known in the food industry and is appreciated in the manufacture of some varieties of cheese. Swiss cheese makers, for example, practice salting heavily those spots on a cheese which show excessive swelling in order to check the formation of gas under that area. Since large loaves of Brick cheese become permeated

with salt more slowly than small loaves, it is logical to assume that salt might have a greater inhibiting effect on gas development in the small loaves of Brick cheese.

Regulation of the size of loaves of Brick cheese is not always easy. Mechanically it is difficult to estimate the amount of free whey in the curd at dipping, and it is practically impossible to measure exactly the amount of curd placed in each mold. Sudden changes in weather cause differences in milk composition and acid development, which in turn influence the yield of cheese per hundred pounds of milk. Effects of such changes are difficult to predict so that it is not surprising to encounter variations in size of loaves. Actually trade demands may require loaves of a certain thickness or weight which might actually favor the occurrence of the splitting defect.

Slight differences in late-gas development were observed in raw- and pasteurized-milk cheese when both lots were made from identical milk. Raw-milk cheese tended to develop somewhat more acid during the manufacturing process than did the pasteurized-milk cheese. When the raw-milk cheese developed the typical splitting defect, the pasteurized-milk cheese showed the same defect very slightly exaggerated by the presence of more of the large sweet holes. Actually, texture grades for these two types of cheese were practically identical.

The results of these early experiments indicated that, although no single factor was wholly effective, it might be possible, by regulating acidity, salt content, and size of the cheese to exercise some measure of control over the defect.

#### DEFECTIVE CHEESE FROM FACTORIES

During the winter of 1936-37, visits were made to a Brick cheese factory which was experiencing considerable trouble with late-gas formation. From 15,000 to 25,000 pounds of milk were being made into cheese each day. Only a portion of the cheese developed the splitting defect; the rest of it was entirely satisfactory and of high quality. The defect was not accompanied by undesirable off-flavor. The factory was visited at weekly intervals for

TABLE 2  
*Relation of average acidity and composition of Brick cheese at two weeks  
of age to late-gas formation*

Degrees of defect*	No. of lots	Acidity	Moisture	Salt	$\frac{\text{Per cent salt} \times 100}{\text{Per cent H}_2\text{O}}$
		<i>pH</i>	<i>%</i>	<i>%</i>	
0	9	5.08	38.5	1.26	3.27
1	6	5.14	38.7	1.27	3.28
2	3	5.16	40.2	1.44	3.60
3	5	5.08	39.7	1.34	3.37
4	9	5.12	39.1	1.27	3.24

\* 0 = none; 1 = slight; 2 = definite; 3 = pronounced; 4 = extreme.

several weeks, and samples of cheese, some of which were split, and some of which were satisfactory, were taken from the curing room at approximately ten days of age. These lots of cheese were scored and analyzed for acidity, moisture and salt. The results of these analyses are shown in Table 2. Of the 32 different lots of cheese examined, nine of them showed no splitting defect; six showed it slightly; nine lots were extremely bad. The analyses in Table 2 were made upon half-inch cross-section slices of each cheese and represent the composition of the cheese as a whole. In general, the average values for acidity, moisture and salt contents were practically the same for satisfactory and defective cheese. Since some of the satisfactory lots were taken from the same vats which produced defective cheese, it seemed possible that the defect might be caused by a treatment following the curd-making process itself.

In nine of the lots of cheese taken from the factory, analyses were made for acidity, moisture and salt on portions of the cheese removed from the center of the loaves. The results of these analyses are presented in Tables 3 and 4. The averages in Table 3 indicate slight differences in composition between the center portions and the cheese as a whole, both in acidity and

TABLE 3

*Relation between late-gas formation and the average acidity and moisture in the whole cheese and in the center portion*

Degree of defect*	No. of lots	Acidity		Moisture	
		Whole cheese	Center	Whole cheese	Center
		<i>pH</i>	<i>pH</i>	%	%
0 .....	3	5.12	5.09	37.9	37.4
1 .....	2	5.12	5.08	39.1	38.4
2 .....	1	5.11	5.11	41.9	39.3
3 .....	1	5.03	4.98	38.3	37.6
4 .....	2	5.08	5.04	38.7	38.2

\* 0 = none; 1 = slight; 2 = definite; 3 = pronounced; 4 = extreme.

TABLE 4

*Relation between late-gas formation and the average salt content of the whole cheese and the center portion*

Degree of defect*	No. of lots	Salt		Per cent salt $\times$ 100 Per cent moisture	
		Whole cheese	Center	Whole cheese	Center
		%	%		
0 .....	3	1.48	1.10	3.92	2.95
1 .....	2	1.39	1.11	3.57	2.90
2 .....	1	1.35	0.97	3.22	2.47
3 .....	1	1.26	1.07	3.28	2.84
4 .....	2	1.18	0.72	3.05	1.86

\* 0 = none; 1 = slight; 2 = definite; 3 = pronounced; 4 = extreme.

moisture. Practically no differences exist between the acidities and moisture contents of the defective and satisfactory cheese. Table 4, however, indicates a generally lower salt content in the most defective cheese and distinct differences between the center portion and the whole cheese. This is even more noticeable when the salt content is stated as per cent of salt in the moisture of the cheese.

The factory operator was advised to increase the salt content of the cheese. Some practical difficulties arose in this connection. The length of time of holding the cheese in the brine could not be increased because the brine tank capacity was already being used to its utmost. The salt concentration of the brine was increased from 75 to 90 per cent saturation as measured by a salt hydrometer. Some of the lots of cheese still showed the splitting defect. This was attributed to the fact that double layers of cheese were floated in the brine tank, and the top layers, naturally, were less exposed to the brine than those beneath. A wooden lattice was made and placed over each layer of cheese as it was put in the brine. The layers were then submerged by weighting down the wooden lattice work. This exposed more cheese to the action of the salt. The defect was still not entirely eliminated, however. Observations of the handling of the cheese in the curing room disclosed the fact that occasionally fresh water from a hose was turned on the cheese after they were taken from the brine tank and while they were on the shelves in the curing room. This rinsing process removed the brine on the exterior of the cheese, reduced the salt content of the cheese and may explain why the defect still appeared from time to time.

These results tended to confirm some of the observations on the influence of salt that were made in 1934-35 and suggested that the typical splitting defect might be developed by special treatments of cheese which would normally be entirely satisfactory.

#### PRODUCING DEFECTIVE CHEESE FROM NORMAL LOTS

In the routine work of the laboratory, a considerable amount of experimental Brick cheese was being produced during the year 1936-37. All lots of cheese were normal and without late-gas defects. In order to verify the earlier observations and the results which seemed to be significant in factory practice, certain loaves of cheese were subjected to some treatment intended to influence the amount of salt in the cheese.

The first experiment consisted of placing a larger portion of curd than usual in the hoops at dipping on three different days in order to make excessively large cheese. Loaves weighing between six and seven pounds were produced in this manner as compared to the normal, which approximated five pounds in weight. Both large and normal cheese were treated in the usual manner accorded the normal cheese. Table 5 shows the results which were obtained when the cheeses were finally analyzed for salt and scored.

TABLE 5  
*Comparison of normal and large-sized cheese*

Lot	Size of cheese	Per cent of salt	Late-gas
1 . . . . .	Normal	2.02	None
	Large	1.10	Definite
2 . . . . .	Normal	1.79	None
	Large	1.02	Many, large, sweet holes
3 . . . . .	Normal	1.91	None
	Large	0.99	Definite

The normal cheese contained more salt than the large cheese. In every instance the normal lots were satisfactory in texture while the large cheese either developed the splitting defect or developed so many large sweet holes that the loaves swelled almost to the splitting point.

In another experiment the amount of salt incorporated in the cheese was reduced by holding the cheese in the brine bath for 24 instead of the usual 48 hours. Typical results from three different days are shown in Table 6.

TABLE 6  
*Comparison of cheese salted 24 to 48 hours*

Lot number	Hours of salting	Per cent of salt	Late-gas
1 . . . . .	48	1.70	None
	24	1.06	Definite
2 . . . . .	48	1.97	None
	24	1.30	Definite
3 . . . . .	48	1.57	None
	24	1.01	Definite

Here again the salt content of the normal cheese is higher than that of the abnormal cheese. In the cheese which was salted for 48 hours, the splitting defect did not occur. In the cheese salted for the shorter period of time, the splitting defect was noted in every instance. In those lots salted for 24 hours, the defect appeared even when the acidity of the cheese was in the range where cheese is criticized for excessive acid development. In those lots where the pH values were well above the acid limits, the splitting defect occurred to a greater degree. One lot of cheese weighing only 3 pounds 7 ounces and containing a relatively small amount of salt developed the splitting defect. The size of the cheese in this instance did not prevent the splitting defect when other conditions favored its occurrence.

#### DISCUSSION

The significance of the results of the experiments with late-gas formation from 1933 to 1935 was so doubtful at that time that they were not published.

Even now the chief reason for summarizing them is to show the reason for later observations and experiments and to emphasize the fact that more than one factor must be involved. Additional evidence of the importance of these other unknown factors is shown, for example, in Table 4 in which are presented much lower percentages of salt in satisfactory cheese than those associated with the splitting defect in the 1933 to 1935 experiments. More evidence of this same type was accumulated in 1936, when about forty factories in all Brick cheese producing areas of Wisconsin were visited at approximately monthly intervals from January until August, and loaves of cheese were brought to the laboratory for scoring and analysis. The frequency distribution of the salt analyses of the cheese is presented in Table 7. The

TABLE 7

*Salt content of commercial Brick cheese collected between January and August, 1936, from 40 Wisconsin factories*

Per cent salt in cheese	No. of samples
1.10-1.29	16
1.30-1.49	27
1.50-1.69	15
1.70-1.89	23
1.90-2.09	18
2.10-2.29	14
2.30-2.49	7
2.50-2.69	4
2.70-2.89	7
2.90-3.00	1

salt content ranges between 1.1 and 3.0 per cent. Observations in this laboratory would indicate that at least one-third of these lots of cheese contained so little salt that they should have been susceptible to the late-gas defect. Obviously, low salt content alone, however, cannot induce the defect if other conditions do not favor excessive gas formation. The reasons why some lots of cheese, despite the relatively low salt contents shown in tables 4 and 7, failed to show the late-gas fermentation are undoubtedly similar to those which determine the appearance of any gas defect in cheese. Small numbers, at least, of the causal organisms can usually be found in milk used for cheesemaking, but there are thresholds of acidity, temperature treatments and moisture contents which definitely limit their development. Such thresholds vary, however, depending upon the number, vitality and activity of the causal organisms. Low salt content, therefore, is regarded as only one, perhaps of several factors, which seem to make cheese more susceptible to the action of the organisms responsible for late-gas formation and splitting of Brick cheese. Certain conditions might be pointed out which seem to induce or to be associated with the development of late-gas, but for which no experimental evidence has been accumulated.

Organisms of the late-gas-forming type are undoubtedly essential for the

production of the defect. These gas producing organisms probably contaminate the milk on the farm, since even the most unusual precautions in the factory failed entirely to check this defect. It is probably true, also, that these undesirable types constitute a greater proportion of the milk flora during the periods when the herds are kept in the barns. When these organisms are present in sufficient numbers there is some reason to believe that the preventive measures mentioned in this discussion might be entirely ineffective. This is illustrated by the data in Table 1. Despite abnormally high salt content in that cheese the defect still persisted.

The general occurrence of the defect during the colder months of the year indicates that possibly the chilling of the curd during the dipping process may be partly responsible for the trouble. When the curd is dipped into the molds, it is customary to fill all the molds at first, then after the curd has settled slightly, the worker returns and adds to the molds sufficient curd to give the cheese its proper size. During this interval, cooling of the curd surface weakens its ability to knit together. Such a condition may even occur in the cooler days of Spring and Summer if the dipping is unnecessarily slow. The fact that the splitting of the cheese always occurs in the horizontal direction might be attributed to this manner of pouring the curd into the molds. It is a fact, of course, that the resistance to swelling is less along the horizontal plane of the cheese.

The common occurrence of the defect in Winter and Spring might be traced to the generally lower temperatures in the draining and salting rooms. Winter milk contains fewer lactic-acid-producing organisms. Factory starters frequently do not supply this need. As a result the cheese curd drains slowly and is frequently too sweet. Low temperatures in the draining room tend to delay necessary acid production. Low temperatures in the salt tank or salting room also tend to decrease salt absorption. It seems possible that such a combination of factors, at a time when contamination is naturally high, might be disastrous. The appearance of the defect in only a portion of the cheese made from the same vat of curd may be explained by variations in:—size of cheese, washing treatments, exposure to salt brine or temperature of cheese during the draining process.

Sometimes during the study of factory-made cheese, excessively large amounts of extraneous material were found. Frequently cow hair, bits of straw, or chaff from grain were found in the split portion of the cheese. The presence of such materials naturally weakened the curd structure and encouraged the splitting of the cheese at those points. It is probably true, also, that these bits of foreign material carried into the cheese excessively large amounts of those organisms responsible for the formation of the gas itself. Obviously, the presence of such foreign material is not to be condoned. A careful maker will not be guilty of permitting such substances

to get into the cheese vat, either through the milk itself or by exposure of the curd in the factory during the dipping operation.

#### SUMMARY

This report indicates that lack of acid development, low salt content and large loaves of cheese tend to encourage the development of late gas in Brick cheese. Although these factors are important they are not necessarily the only ones involved in the production of this defect.

# SEEDING TEST FOR CRYSTALLINE BETA LACTOSE

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## INTRODUCTION

Many times it is of great importance to know whether the lactose in a dried milk product is present as a glass or is in the crystalline state, and if in the crystalline state whether the crystals are alpha hydrate, beta anhydride, or both. This information can be obtained rather easily by taking advantage of the property of lactose to form fairly stable supersaturated solutions. Below 93° C., supersaturation with respect to alpha lactose hydrate predominates, and above this temperature, supersaturation with respect to beta lactose anhydride. If the concentration is so controlled that the solution is supersaturated with respect to one form and is undersaturated with respect to the other forms of lactose, advantage can be taken of the supersaturated state of the solution to test, by seeding, for the presence in the crystalline state of the form of lactose with respect to which the solution is supersaturated.

## SEEDING TEST FOR CRYSTALLINE ALPHA LACTOSE HYDRATE

Hudson and Brown (1) described a test for crystalline alpha lactose hydrate based on this principle. They cooled from 20° C. to 0° C. a solution saturated with lactose, with the forms in equilibrium at 20° C., and used the solution for testing beta lactose preparations which were suspected of containing crystalline alpha lactose hydrate. Since crystallization proceeds slowly at 0° C., and such a temperature is not conveniently obtained in the laboratory, Troy and Sharp (2) used a modification of the test which is much more sensitive and convenient. A solution of lactose saturated at 50° C. with the forms in equilibrium was filtered, and cooled to 20° C. To each of several 10 ml. portions of this solution, in test tubes, was added about 25 mg. of test sample, and the tubes were stoppered and shaken. If alpha lactose hydrate crystals are present, the solution becomes turbid in about 15 minutes. If the solution remains clear for about one hour, the absence of alpha lactose hydrate crystals is indicated. Some idea of the amount of alpha lactose hydrate crystals or nuclei can be obtained by the time required to induce crystallization and by the degree of turbidity of the solution. The test is sensitive, and care in handling the solution is necessary to prevent contamination with crystalline alpha hydrate crystals; on the other hand a few perfect alpha lactose hydrate crystals when present may not induce copious crystallization of the solution. Dehydrated alpha

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lactose hydrate also induces crystallization, because it hydrates before it dissolves, and the characteristic arrangement of the molecules of the sugar is not altered by simple removal of the molecules of water. Beta crystals and lactose in the glass form do not induce crystallization.

#### SEEDING TEST FOR BETA LACTOSE CRYSTALS

A seeding test for beta lactose anhydride crystals, based on the same principle, has been used in this laboratory for a number of years, and several hundred samples have been tested. In carrying out the test it is necessary that a solution supersaturated with respect to beta lactose and undersaturated with respect to alpha lactose hydrate be prepared and maintained for a period of time sufficiently long to determine whether crystallization can be induced by adding a small portion of a product suspected of containing beta crystals.

The test is carried out by means of apparatus of the type illustrated in

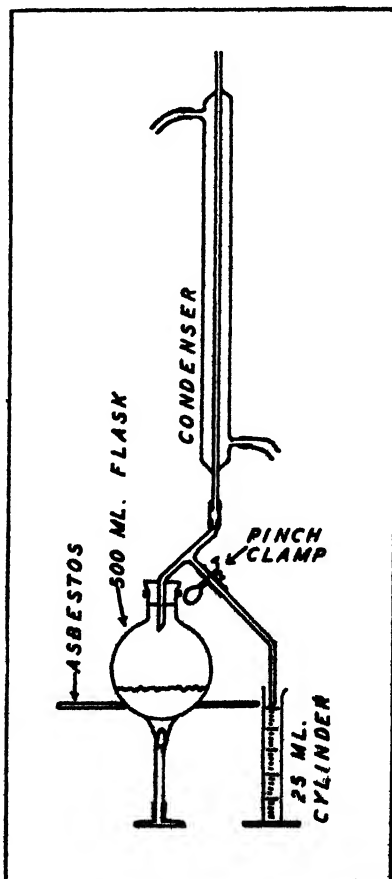


FIG. 1. Apparatus used for detecting beta lactose crystals in a product by its seeding effect on a solution supersaturated with respect to beta lactose.

Figure 1. A 500 ml. short-necked, round-bottom flask is attached to a condenser by means of a bent T tube, the stem of which is connected through rubber tubing and a pinch clamp to a 25 ml. graduated cylinder. It is possible to operate this apparatus either for refluxing or for the removal of solvent by distillation, by closing or opening the by-pass. No spattered solution should be permitted to dry on the inner walls of the flask above the surface of the solution, because this material will contain crystals of both alpha lactose hydrate and beta lactose. These crystals will eventually wash down into the solution and will induce crystallization. The drying is prevented by an asbestos guard  $\frac{1}{4}$  inch thick, with a round hole of such a size that no direct heat from the flame is permitted to come near the surface of the boiling syrup.

To prepare the solution for the seeding test 58 ml. of water is added to 100 grams of alpha lactose hydrate in the flask, which is attached to the condenser in the refluxing position. In the early stages of heating the contents of the flask must be agitated by shaking to prevent the burning of the lactose. The solution should be boiled with the condenser in the refluxing position until all of the crystals have disappeared. The by-pass is then opened and 20 ml. of water is removed by distillation; the by-pass is then closed and the condenser is operated in the refluxing position. The portion of the T tube above the pinch clamp traps about 1 ml. of water. The solution in the flask is now supersaturated with respect to beta lactose anhydride and is undersaturated with respect to alpha lactose hydrate.

To carry out a test the stopper is removed and about 25 mg. of the material to be tested is knocked from a spatula into the flask. The stopper is then quickly replaced, and refluxing is continued. If the test material contains beta lactose crystals, the solution will become turbid in about 2 to 3 minutes. If the solution remains clear at the end of 10 minutes, the absence of beta lactose anhydride crystals is indicated. If the first sample tested does not induce crystallization at the end of 10 minutes, another sample can be added and the process continued until a sample is encountered which induces crystallization. After beta crystallization has been induced, it is necessary to reestablish the state of beta supersaturation. This is done by pouring down through the condenser the 20 ml. of water in the graduate. This dissolves the beta crystals in the flask. Again 20 ml. of water is distilled off, and the solution is then ready for further tests. The same solution can be used for about 2 hours. When a number of samples are to be tested, a battery of two or more testers may be operated at once. The testing can be shortened further by adding material from two or three samples at once to each flask. If at the end of 10 minutes crystallization is not induced, the absence of beta crystals in all of the samples is indicated. If crystallization is induced, then the samples must be re-tested individually to identify the specific ones containing beta crystals.

## APPLICATIONS

*Lactose.* This method was first developed for testing products made in a study of methods of preparing beta lactose, particularly in relation to the importance of seeding, temperature, and the rate of removal of water from the solution as influencing the character of the product obtained.

It is difficult to prepare beta crystals entirely free from alpha crystals. The reason for this is that the beta lactose is crystallized from solution. The last trace of mother liquid or of the solution of lactose in the wash water adheres to the surfaces of the beta crystals. When the last bit of water is removed by drying, the water is removed faster than the forms can change and crystallize; consequently the solution becomes supersaturated with respect to both the alpha and the beta form, and they both crystallize out together on the surface of the beta crystals. Seeding tests, optical rotations, and moisture tests have indicated that this is true. A similar crystallization was found to occur on the surface of alpha lactose hydrate crystals. A number of samples of commercial alpha lactose hydrate were tested and they all gave tests indicating the presence of beta crystals. If, however, the crystals were moistened with a little water before being added to the test solution, crystallization was not induced. This small amount of water dissolved the beta crystals present on the surface of the alpha crystals before they were added to the supersaturated beta solution, and consequently crystallization was not induced. This demonstration of the presence of beta lactose crystals on the surface of ordinary alpha hydrate crystals raises an interesting point as to the purity of the alpha lactose used by investigators.

*Dried milk.* The samples tested included milk dried by the pressure and centrifugal spray, the vacuum and open roll, and the flake and old Campbell processes. In the nearly one hundred tests which have been run on freshly prepared products and products even ten years old which have been maintained at a low moisture content, the tests for beta lactose crystals have been negative.

If, however, the dried milk has been allowed to take up moisture and cake, the tests for beta lactose are usually positive. The lactose glass of the dried milk is diluted by the absorption of moisture to a concentration at which crystallization can occur; and since the solution is highly supersaturated with respect to both the alpha and the beta forms, under some conditions of time and moisture content both the alpha hydrate and the beta anhydride crystallize out together at room temperature.

*Dried whey.* Most of the methods of drying whey yield products in which beta crystals are absent. This includes: (1) the ordinary spray drying (glass); (2) partial drying by spraying (glass), maintenance of the product in a moisture-reinforced atmosphere (glass to alpha hydrate) followed by further drying; (3) spray drying (glass), rewetting, holding (glass to alpha hydrate) and finally redrying; and (4) evaporation to high

solids content, withdrawing from the evaporator, holding for several hours (supersaturated solution to alpha hydrate) and finally drying by tunnel methods. The greater hygroscopic properties of the dried whey probably accounts for the failure of the beta form to crystallize on caking in a similar manner to dried milk.

A new procedure for atmospheric roll drying has recently been developed which is continuous, does not involve a holding period, and results in a product in which the lactose is present largely as beta crystals. All samples of this product tested gave positive seeding tests for beta crystals.

*Ice cream.* A number of samples of ice cream were tested and none of them gave a positive test for beta crystals.

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# PHYSICAL STATE OF LACTOSE AS INFLUENCING THE DETERMINATION OF MOISTURE IN DRY MILK PRODUCTS BY THE TOLUENE DISTILLATION METHOD

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## INTRODUCTION

The measurement of the volume of water removed from a product by suspending it in an oil or an organic liquid which is immiscible with water, heating above the boiling point of water, distilling, and condensing the water, is frequently the most satisfactory method for the determination of moisture. In some procedures the liquid used is a nonvolatile oil the temperature of which is maintained considerably above the boiling point of water. This method is unsuitable for materials which decompose at the high temperatures necessary for the rapid removal of the water. Therefore a liquid with a boiling point slightly above that of water is selected, and both water and the immiscible liquid are distilled together, the lower temperature reducing decomposition by heat and the volatilization of the immiscible liquid aiding in sweeping out the water vapor. The vapors are condensed, the water is trapped out and measured, and the immiscible liquid is returned automatically to the distilling flask for redistillation. A method of this type was described by Dean and Stark (3), using xylene (boiling point 138–142° C.) as the immiscible liquid. Bidwell and Sterling (2) substituted toluene (boiling point 111° C.) for xylene, and modified the apparatus slightly to make it more suitable for food products. They used it for the determination of the moisture in a number of products, among them dried milk. Jones and McLachlan (6) preferred toluene to benzene or petrol as the immiscible liquid for the moisture determination on a number of food products. Wright (13) found the toluene distillation method more suitable than oven drying, for the determination of moisture in dried milk, and recommended a two-hour distillation time. The toluene distillation method has since met with general favor for the determination of moisture in dried skimmilk, and has been recommended by the Committee on Methods, of the American Dry Milk Institute (10) (11). Thompson and Fleming (9) made a careful study of the moisture values obtained by the toluene method and the method recommended by the Association of Official Agricultural Chemists (1). The latter consists in drying a 1.0–1.5 gram sample for 5 hours in a vacuum (less than 100 mm.) oven at 100° C., during which time two bubbles per second of dried air are

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admitted. The toluene distillation method was found to be the more satisfactory by Thompson and Fleming.

Dried skimmilk is half lactose, and investigators have shown a tendency to consider that the removal of moisture from the lactose in dried skimmilk would follow a course similar to the removal of water from crystalline  $\alpha$  lactose hydrate. Troy and Sharp (12) and Sharp (8) have shown that the lactose in spray dried, atmospheric roll dried, and vacuum roll dried milk powder is not crystalline but is present as a glass. Crystalline  $\alpha$  lactose hydrate is present, however, in milk dried by the flake or old Campbell process, and in milk dried by the other methods after caking. Therefore a study of the removal of the water of crystallization from  $\alpha$  lactose hydrate has a direct bearing only on the determination of moisture in these types of dried milk.

Dried whey has recently been produced in which a considerable part of the lactose is present as crystalline  $\beta$  lactose. Therefore the lactose is present in three different physical states in the different types of dried milk and whey, namely: as a glass, as the crystalline  $\alpha$  hydrate, and as the crystalline  $\beta$  anhydride. The rate of removal of moisture from lactose is influenced by its physical state.

#### EXPERIMENTAL

The moisture in a series of samples of dried whey was determined by oven drying. The samples varied in lactose content, in the physical state of the lactose, and in acidity. Dried wheys show the differences in the physical state of the lactose more strikingly than dried skimmilks, because the amount of lactose present is greater. An air oven was used, as well as two types of vacuum ovens which differed in the degree of vacuum and in the method of applying the heat. The time of drying and size of sample were varied. In addition, moisture was determined by the Mojonnier procedure (Mojonnier and Troy (7)) which involves weighing the sample, dissolving it, drying it quickly, and weighing. In this procedure, the lactose is dissolved, and thus all forms are converted to the same physical state and dried as a glass. We found it difficult to get satisfactory duplicates when applying this method to dried whey. Darkening was variable and often considerable when using this method.

Since some of these samples contained crystalline  $\alpha$  lactose hydrate, a sample of about 160 mesh  $\alpha$  hydrate was included each time with the other samples. A method, to be satisfactory for all samples, should remove all of the water of crystallization from the  $\alpha$  lactose hydrate in order to avoid difficulties in checking and agreement between duplicates, and to express the moisture content of the various types of products on a comparable milk solids basis. It will be observed that the removal of the water of crystallization was complete or nearly complete in only a few cases.

Results obtained are reported in Table 1. This table also gives results

TABLE 1  
Moisture content of dried whey as determined by several different procedures

Whey sample no.	Total anhydrous lactose in whey	Fraction of total lactose as alpha (anhydrous)	pH	Vacuum oven pressure 1 mm. Central Scientific oven						Vacuum 27 in. Mojonnier		Air oven	Mojonnier dissolved	Toluene
				5 gm. 85° C. 5.5 hrs.	5 gm. 85° C. 22 hrs.	5 gm. 85° C. 41 hrs.	2 gm. 85° C. 17 hrs.	2 gm. 100° C. 3 hrs.	2 gm. 100° C. 16 hrs.	2 gm. 100° C. 5 hrs.	5 gm. 100° C. 15 hrs.	2 gm. 100° C. 15 hrs.	0.3 gm. 100° C. ½ hr.	50 gm. 111° C. 1 hour
1	65.8	93.2	4.8	% (4)	% (5)	% (6)	% (7)	% (8)	% (9)	% (10)	% (11)	%* (12)	%* (14)	%** (15)
2	60.8	98.2	4.6	2.80	4.60	4.98	4.73	4.54	5.35	5.25	7.30	8.05	4.60	5.1
3	63.3	89.6	4.2	2.20	3.99	5.16	4.04	3.64	6.03	6.06	9.75	13.74	4.53	4.5
4	64.6	90.1	5.0	2.77	5.21	6.37	5.23	5.10	7.74	7.84	10.54	13.56	5.42	4.3
5	70.9	91.4	5.8	2.14	4.06	4.82	4.30	3.56	5.04	5.13	5.58	6.93	4.63	4.5
6	58.4	93.2	4.9	1.58	3.80	4.71	3.96	2.89	4.86	4.86	6.78	8.85	4.26	3.6
7	64.6	22.6	4.5	2.52	3.86	5.10	3.84	3.32	6.85	7.06	10.61	13.60	5.08	5.5
8	65.8	19.5	4.4	2.31	2.58	2.80	2.55	2.90	3.65	3.77	7.54	8.36	3.32	4.2
9	60.2	21.1	3.9	2.08	2.42	2.72	2.47	2.92	3.54	3.64	5.90	8.37	2.10	2.8
10	68.3	24.5	4.3	2.32	2.65	3.08	2.63	2.75	4.12	4.33	7.41	8.49	3.04	3.2
α lactose hydrate	95.0	100	...	1.75	1.94	2.13	1.81	1.85	2.63	2.38	3.41	5.43	1.34	2.3
				.82	2.71	4.50	2.86	1.92	4.80	4.95	5.01	2.22	3.87	4.2

\* Duplicates not satisfactory.

\*\* Before shaking during distillation was adopted.

obtained with the toluene distillation method. At the time these tests were made the technique of applying the toluene distillation method to dried wheys was rather unsatisfactory because of the settling out of the product, and burning. Later, when the toluene procedure was modified to give satisfactory results with dried whey, our supply of these samples was exhausted.

The results presented in Table 1 are variable and on the whole are not satisfactory. Moisture as determined by the different procedures does not always arrange the samples in the same order with respect to moisture content. Only in procedures (9), (10), (11), and (12) was the water of crystallization removed satisfactorily from the  $\alpha$  hydrate. Some of the samples subjected to procedures (11) and (12) darkened greatly. The darkening was associated with high apparent moisture content. Procedure (9)-(10) was perhaps the best from the standpoint of satisfactory duplicates. Procedure (8) most nearly approaches the A.O.A.C. method, but it does not remove the moisture satisfactorily from the  $\alpha$  hydrate. The vacuum was higher and the drying time shorter than in the A.O.A.C. method.

#### MODIFIED TOLUENE DISTILLATION

The Bidwell-Sterling type of apparatus was used with 50 gram samples and 120 ml. of toluene. Difficulty with burning on the bottoms of the flasks, due to settling, was encountered when the method was applied to dried whey. To prevent settling, the necks of the distilling flasks were firmly clamped to a long board which was suspended horizontally. One end of this board was connected to a motor-driven eccentric which gave a thrust of about  $\frac{1}{4}$  inch, and operated at a speed of three or four revolutions a second, shaking the flasks violently during the distillation. Using this procedure, the maximum difference between duplicates rarely exceeded .2%. All of the determinations which follow were carried out using this method. The amount of water removed was measured at 10 minute intervals; the water adhering to the sides of the tube and condenser was loosened with a long wire before each reading. The toluene distillation method has the distinct advantage that the moisture-time relationship can be determined easily. This gives an insight into the factors contributing to the moisture content.

#### CRYSTALLINE LACTOSE

As expected, the rate of removal of water from crystalline  $\alpha$  lactose hydrate depends upon the size of the crystals. This was shown for oven drying, by Herrington (5). It is equally true when the moisture is removed by the toluene distillation procedure, as is shown in Figure 1. Alpha lactose hydrate contains 5% of water of crystallization. A number of samples of dried whey and dried milk which contained  $\alpha$  lactose hydrate crystals were suspended in a saturated lactose solution on microscopic slides. A microscopic examination indicated that some of the largest  $\alpha$  lactose hydrate

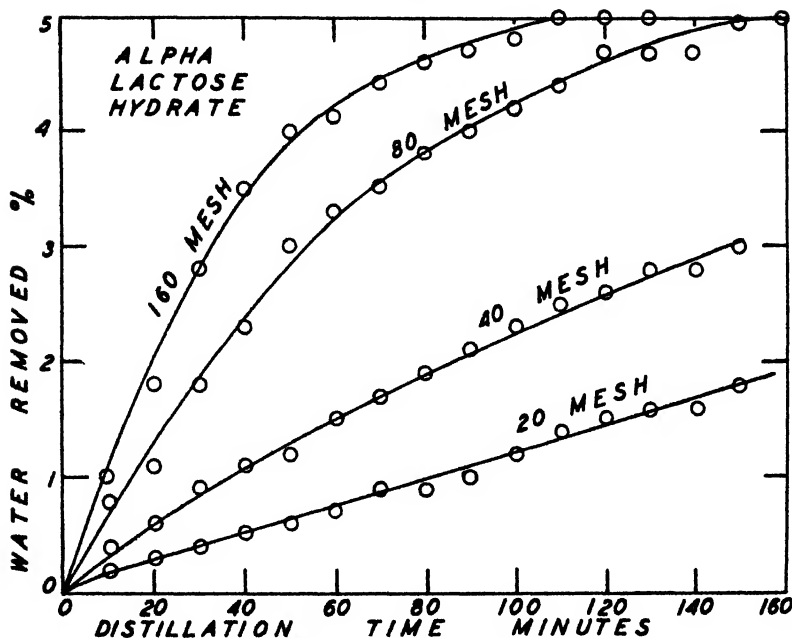


FIG. 1. Effect of the size of  $\alpha$  lactose hydrate crystals on the rate of removal of moisture by the toluene distillation method.

crystals corresponded in size to the largest found in the 160 mesh lactose of Figure 1. Thus, a two-hour distillation time would be sufficiently long to remove the water from crystalline  $\alpha$  lactose hydrate present in any ordinary dried whey or dried milk. The amount of moisture removed from the crystalline  $\alpha$  hydrate increased with each ten-minute increment of distillation time up to 110 minutes for the finer crystals. The curves indicate a slow removal of moisture from the crystalline  $\alpha$  hydrate. Pure  $\beta$  anhydride crystals gave no moisture when subjected to two hours of toluene distillation.

#### DRIED CASEIN

Quantitatively dried skimmilk is mainly an intimate mixture of dried lactose and dried casein. Consequently, moisture-distillation time curves were determined using several samples of dried casein. The results are presented in Figure 2. Here again size of particle was a factor in the rate of removal of moisture. With samples of 60 mesh, the break in the curve is fairly sharp, indicating that most of the moisture is removed in the first 60 minutes. Since the particles of casein are small in dried milk, these results indicate that any pronounced delay in removal of moisture from dried milk could hardly be attributed to the casein. This figure indicates that the toluene distillation method would be satisfactory for the determination of moisture in dried casein.

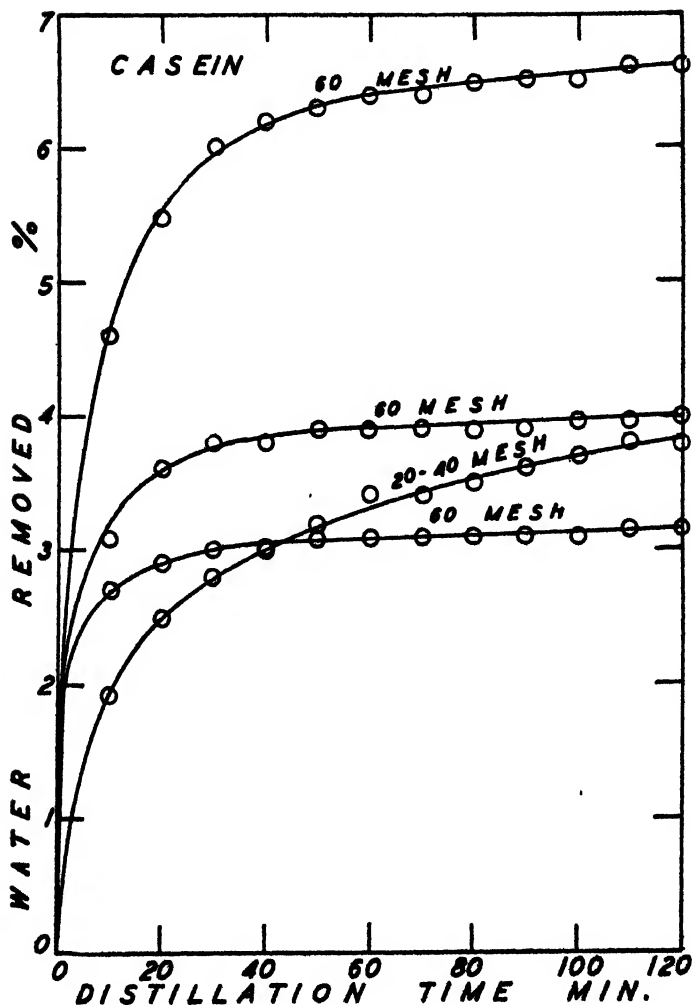


FIG. 2. Moisture content of commercial casein as determined by the toluene distillation method.

#### DRIED SKIMMILK

Lactose is present in the glass state in the fresh, spray dried, and roll dried skim milks, and the curves presented in Figure 3, indicate that most of the moisture is removed from these types of dried milk in the first 60 minutes. This result is in agreement with the previous reports (9) (10) (11). For the samples prepared by the flake and old Campbell process, however, 60 minutes of distillation is not sufficient to remove all of the moisture. The shape of the curves is quite different because much of the lactose is present in the form of crystalline  $\alpha$  hydrate. Approximately two hours of distillation is necessary for moisture determinations on this type of dried milk.

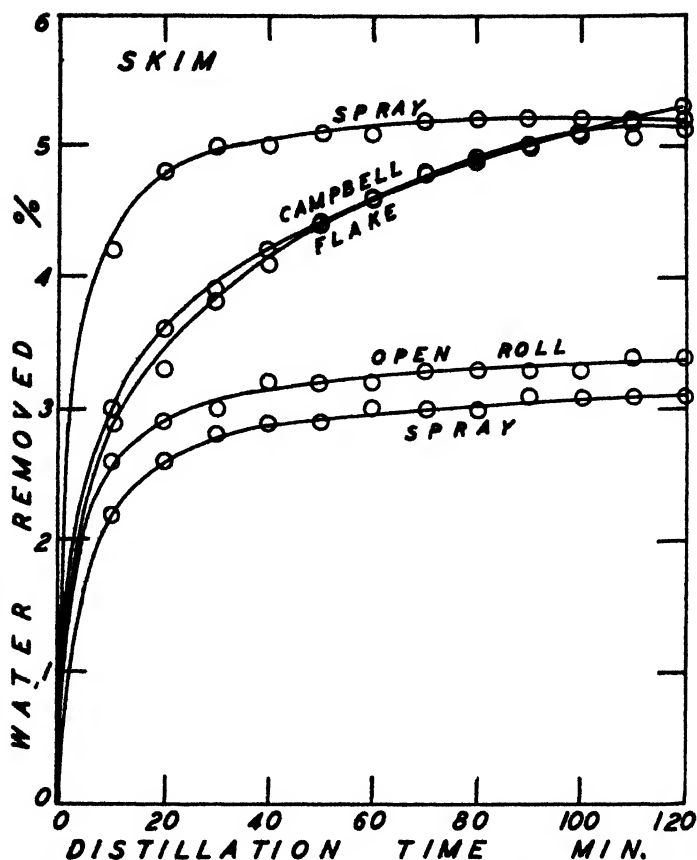


FIG. 3. Effect of the presence of crystalline  $\alpha$  lactose hydrate (Campbell and Flake) on the rate of removal of moisture from dried skim milk. Toluene distillation method.

#### DRIED WHEY

Typical moisture-distillation time curves for dried whey are given in Figure 4. The curves broke sharply with wheys in which the lactose was present as a glass or as crystalline  $\beta$  anhydride. Practically all of the moisture was removed in 60 minutes. The moisture was removed much more slowly from samples in which the lactose was present as  $\alpha$  hydrate crystals, and a two hour distillation time was necessary for a moisture determination. The samples of whey in which the lactose was present as the  $\alpha$  hydrate darkened during the distillation, and moisture, perhaps resulting from decomposition, continued to be given off slowly even when the distillation time was prolonged to three hours. The samples in which the lactose was present as a glass or as crystalline  $\beta$  lactose were a light straw color at the end of two hours.

The moisture content of spray dried whey (lactose in the glass state) cannot be determined by the toluene distillation method if the moisture content exceeds 3.5–4.0 per cent. Because of the syrupy state of the lactose,

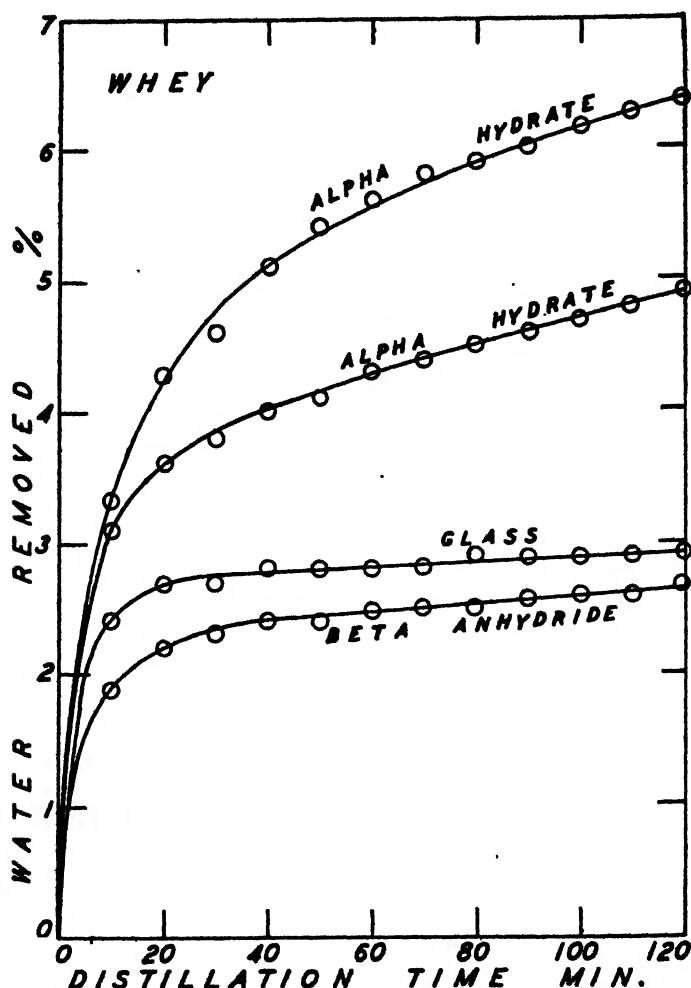


FIG. 4. Effect of different physical states of the lactose on the rate of removal of water from dried whey by the toluene distillation method.

such wheys when heated to the temperature of the boiling toluene form a sticky, doughy mass.

#### BEFORE AND AFTER CAKING

Troy and Sharp (12) showed that the caking of dried milks which occurs when they absorb moisture is due to the crystallization of the lactose as the  $\alpha$  hydrate. In the ordinary spray dried or roll dried products the lactose is present in the non-crystalline state as a concentrated syrup or glass. This glass is very hygroscopic, and it will absorb moisture from atmospheres of low relative humidity. The absorbed moisture dilutes the lactose solution. The particles then become sticky, and in the case of spray dried whey plastic masses are obtained. This dilution of the syrup permits a freer movement

of the molecules and crystallization occurs, mainly as the  $\alpha$  hydrate. This crystallization produces a hardening of the mass, which is called caking. After this process has been completed the material may be ground to a fine powder which ordinarily will not cake again and will not become sticky unless exposed to a rather high relative humidity.

Samples of spray dried whey and open roll (previously ground to reduce its bulk so that 50 gram samples could be distilled) and spray dried skim milk were divided into aliquots and one set was placed for ten days at a relative humidity of 80 per cent. During this time the material took up water, became sticky, and caked. These samples were then held for three days at a relative humidity of 10 per cent. They were then ground, and held at a relative humidity of 20 per cent for two days. Moisture determinations were then made on the aliquots. The results are shown in Figure 5. The aliquots which had not been permitted to absorb moisture gave the relatively sharp breaks in the curves characteristic of such products. The aliquots which had

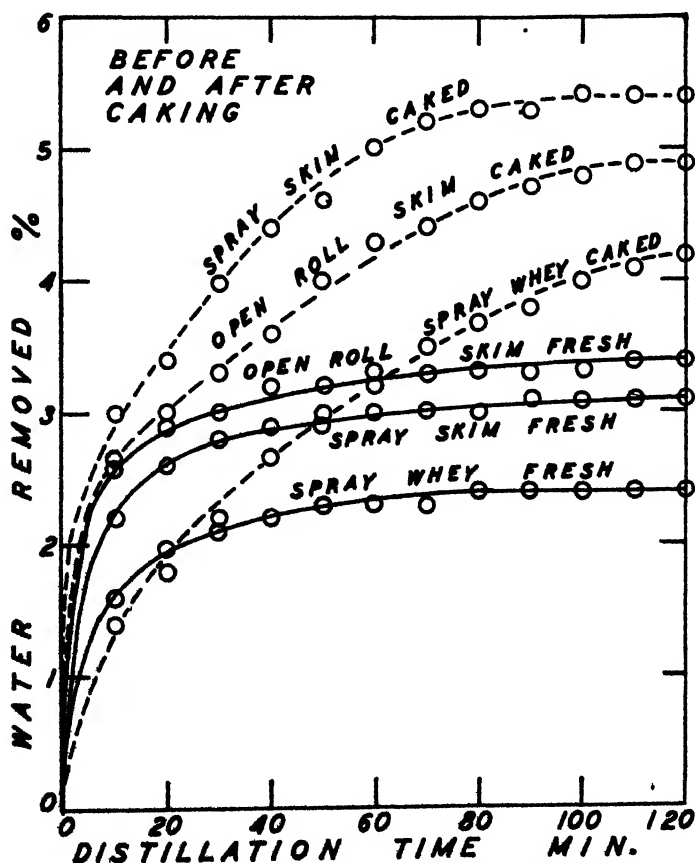


FIG. 5. Effect of previous caking on the rate of removal of moisture from dried milk and dried whey by the toluene distillation method.

gone through the caking process gave curves characteristic of products containing crystalline  $\alpha$  lactose hydrate, and similar to those obtained with the flake or old Campbell process dried skimmilk and similar types of dried whey.

#### DISCUSSION

The determination of moisture in most food products is arbitrary, often to a disconcerting degree, and results which check are obtained only when the complete details of the method are adhered to rigorously. In oven drying the position of the samples in the oven, as well as the number of samples run at one time, often influences the results. Several different moisture-holding or yielding materials are present in many products and the moisture is held by several different mechanisms. In the removal of moisture by dry heating, it is often difficult to tell when a change from one moisture-holding mechanism to another occurs, and when what might be called the "true" moisture is removed without at the same time producing a change in weight of the product and the formation of water due to chemical decomposition.

The main moisture-holding materials in dried skimmilk are the proteins, principally casein, the salts, and the lactose. All of these materials have somewhat different water-holding properties. The water is apparently removed fairly readily from the casein if it is present in a fine state of division. The removal of water from the salts present in dried skimmilk probably involves no difficulty, but the salts present in dried whey, particularly whey which has previously undergone lactose fermentation or which has been highly acidified before drying, may exert a definite influence on the moisture determination. The fermentation products alter the water-holding capacity and the extent of decomposition during the heating.

The amount and physical state of the lactose exerts a marked influence upon the rate of removal of moisture. In order to make a true comparison of the different types of dried milk and dried whey on the basis of their content of milk constituents, the water of crystallization should be removed in the moisture determination from those products containing crystalline  $\alpha$  hydrate. If the lactose is present as a glass, the methods for determination of moisture do not remove quite all of the moisture, but they reduce the moisture content of the glass to a constant, very small amount (4) (12).

Great variations in apparent moisture content resulted when samples of wheys of different characteristics were dried by heating in ovens. The degree of pressure reduction exerted a marked effect upon the results. As the pressure was reduced, the apparent moisture content increased, passed through a maximum, and then decreased again at very low pressures. These results mean that the so-called degree of vacuum under which the samples are dried has a marked effect upon the apparent moisture content, particularly of some types of whey, and makes drying by vacuum oven procedures uncertain unless the pressure in the drying oven is accurately controlled to specific,

preferably very low values. A.O.A.C. method simply says less than 4 inches (100 mm.).

The method of applying heat to the sample in the vacuum oven also influences the rate of removal of moisture. In one oven used, the sample was heated largely by radiation, in another type, by conduction. Experience indicates that the apparent moisture contents are higher in the oven in which the heat is transmitted by conduction.

Accurate moisture loss-time curves are not easily obtained by oven drying. Moisture-time curves are important because they give an indication of the properties of the material from which moisture is being removed.

The toluene distillation method was more satisfactory than oven drying for the determination of the moisture in dried whey. The equipment required is relatively simple and cheap. The method is readily adapted to control laboratory or plant use. Large samples are used. Duplicate results usually check within .1 per cent. Furthermore, distillation time curves are readily obtained which reveal the characteristics of the materials from which moisture is being removed. In adapting the toluene distillation method to the determination of moisture in dried whey, violent shaking during the distillation was found to be necessary for satisfactory results.

#### SUMMARY

1. Dried skimmilk and dried whey containing lactose in the crystalline  $\alpha$  hydrate form require appreciably longer heating times for the removal of moisture than do similar products containing the lactose in the glass or crystalline  $\beta$  anhydride form.

2. Determination by oven drying, of the moisture content of different types of dried whey, presents considerable difficulty due to the differences in acidity, amount of fermentation products, per cent and physical state of the lactose, and method of heating and degree of vacuum in the oven.

3. The toluene distillation method possesses distinct advantages for the determination of the moisture in dried milk, dried whey, and dried casein. The moisture loss time relationship can be determined without interrupting the process or interfering with the determination. The moisture loss time relationship indicates the completeness of removal of moisture, and often indicates whether more than one mechanism is involved in the loss of moisture.

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# THE EFFECT OF GELATIN ON THE CURD TENSION OF MILK

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Early in 1932 we attempted to find a method whereby a soft curd milk for use in infant feeding could easily be obtained at low cost. At that time natural soft curd milk was available in relatively few sections of the country; moreover, the expense of production placed it beyond the means of many. Our work on the modification of milk had several interruptions, but has now been resumed. While some phases of the subject require further investigation, the results thus far are, we feel, of sufficient interest to warrant publication at the present time.

No attempt will be made here to review the various methods of producing soft curd milk. Most of them possess, however, certain disadvantages. Milk which has been rendered soft curd by special processes, such as homogenization, can be obtained with difficulty or not at all by those living in isolated sections of the country. In addition, the possibility exists that soft curd milk, in which the calcium equilibrium has been altered by the zeolite treatment, may have a calcium content below the optimum for that required by those infants whose mechanism for the utilization of calcium is inefficient. The energy value of natural soft curd milk is low. Weisberg, Johnson and McCollum (11) and Hill (6) reported that natural soft curd milk contained less casein, calcium and phosphorus than hard curd milk. Berry (1) found that rats showed larger gains in body weight on a hard curd milk, which had been rendered soft curd by viscolization, than on a natural soft curd milk.

In view of these facts, it would seem highly advantageous to find a method for the preparation of soft curd milk by the addition of some easily procurable substance which would in itself add to the nutrient value of the milk. The use of cereals, such as barley flour, which have been employed in infant feeding, is prohibited in cases of infants with low carbohydrate tolerance. The fact that breast milk, which has a low curd tension, has a much higher albumin: casein ratio than does cow's milk, suggests the use of a soluble protein in the preparation of soft curd milk. In the pages that follow we will describe some of our experiments on the effect of gelatin on the curd tension of milk.

## THE RELATION BETWEEN CURD TENSION AND THE CONCENTRATION OF PROTEIN IN MILK

### A. Casein

The hardness of the curd formed in the clotting of milk must obviously bear some relation to the concentration of the casein in that curd. But, in a

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system as complicated as milk, several other components must exist which exert more or less influence on the character of the curd. It is clear, therefore, that in order to determine the exact relationship of the curd tension to the concentration of the casein, both the nature of these other components as well as the extent of their influence must be known. Even though an attempt is made to maintain a fairly constant concentration of these components in the milk, the results will at best give one but a rough idea as to the extent of the dependence of curd tension on casein concentration.

The casein content of several samples of raw whole milk<sup>1</sup> was determined by the official method (8). The milks were all obtained from Guernsey or Jersey cows, except in a few cases of soft curd milk, where a pooled sample was used. The sealed bottles containing the milk were packed in ice and sent from the dairy farms in New Jersey or New York State to our laboratory in New York City. The curd tension of these same milks was determined by the method of Hill (5). The concentration of pepsin was constant in all the experiments in which curd tension was determined; the addition of the large quantity of calcium chloride in the coagulation probably resulted in a fairly constant concentration of calcium ions, while the high buffer capacity of the system helped to keep the concentration of hydrogen ions within rather narrow limits. Our results, as well as those of Morris and Richardson (7), of Weisberg, Johnson and McCollum (11), and of Doan and Welch (3) are

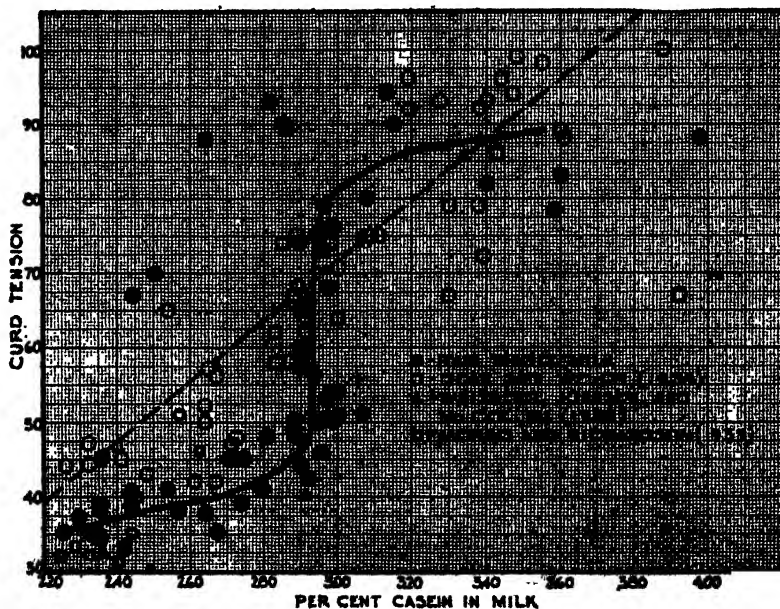


FIG. 1. Relation between the concentration of casein and the curd tension of milk.

<sup>1</sup> We wish to thank the Sheffield Farms Company for supplying the milk used in part of this research.

shown in Figure 1. Most of the results of Espe and Dye (4) fell outside the limits of curd tension in the figure. The variation in the curd tension of different milks with approximately the same concentration of casein shows clearly the influence of other factors in the coagulating system. A large number of our points, however, appear to describe an S-shaped type of curve. The straight line was drawn from the Doan and Welch equation (3), which relates curd tension to casein concentration. Our values seem to follow the S-shaped curve rather better than the straight line.

In most of the milk samples thus far investigated a high curd tension is accompanied by a high casein concentration, and a low curd tension is found in milks with a low casein concentration. However, wide variations in curd tension occur in milks with the same concentration of casein. Further investigation is necessary before the exact relation between curd tension and casein concentration can be ascertained.

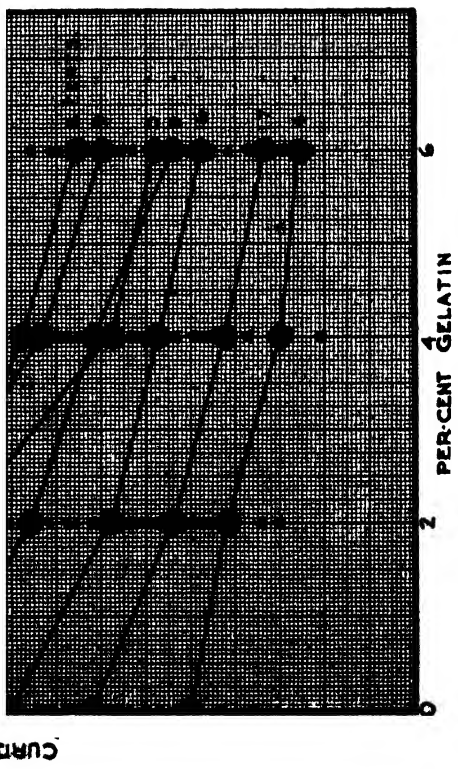
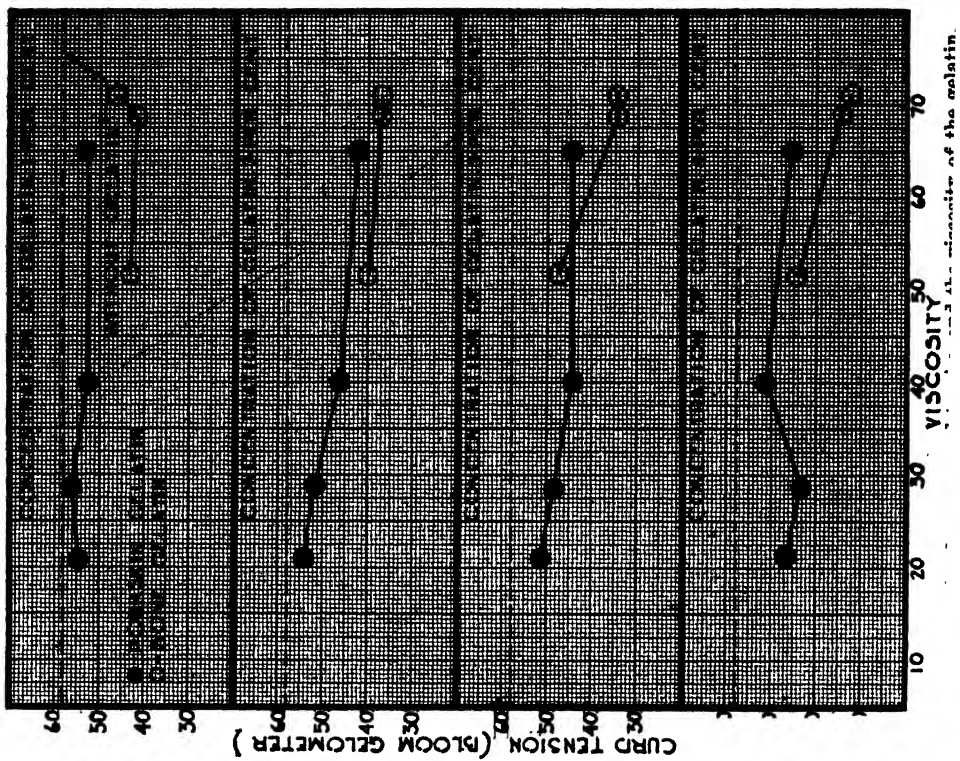
### B. Gelatin

It has just been shown that in a large number of cases the curd tension of milk increases with increasing concentration of casein. The effect of a soluble protein, gelatin, on the curd tension of milk will now be described.

The gelatin used in this work, unless otherwise noted, was a sample of Knox Gelatine.<sup>2</sup> The milk samples were the same as those described in the preceding section. The gelatin was weighed into the regular test jar, 100 cc. of milk at room temperature was introduced, and the mixture was allowed to stand for a few minutes until the gelatin was thoroughly soaked. The jar was then placed in a water bath at 60° C. and the contents were stirred until the gelatin was dissolved. The mixture was then allowed to stand at room temperature with occasional stirring until the temperature reached 35° C., when the curd tension was determined by the method of Hill. Milk, which contained no gelatin but which was subjected to the same conditions of heating, exhibited the same curd tension as the unheated milk. The results are given in Figure 2.

Each of the curves represents the average values obtained over a range of about 10 grams of curd tension; thus, the curve which begins at a curd tension of 45 grams is the average of all the results for milks with an initial curd tension between 41 and 50 grams. The curves illustrate in striking fashion the lowering of the curd tension of milk by gelatin. If a curd tension of 33 grams or lower is taken to represent a soft curd milk, then, in general, milks with an initial curd tension up to about 50 grams are converted into soft curd milks by the addition of two per cent of gelatin, while those with an initial curd tension up to about 70 grams are rendered soft curd by the addition of four per cent of gelatin. Most of the samples of

<sup>2</sup> Bone gelatin: pH 6.1, viscosity 61, jelly strength 200.



milk, which we have purchased in the open market in New York City and elsewhere have had curd tensions below 50 grams.

The percentage reduction in curd tension by different concentrations of gelatin is shown below in Table 1.

TABLE 1  
*Percentage reduction in the curd tension of milk by gelatin*

Range of initial curd tension	Average percentage reduction in the curd tension of milk by gelatin of the following concentrations		
Grams	2 per cent	4 per cent	6 per cent
18-30	14	37	44
31-40	27	41	54
41-50	25	35	47
51-60	21	34	49
61-70	24	49	57
71-80	32	42	50
81-94	38	53	60

In general, the percentage reduction in the curd tension of milk by two per cent of gelatin is greater for a hard curd than for a soft curd milk. The addition of the first two per cent of gelatin to the milk brings about a greater percentage reduction than do subsequent additions of two per cent of gelatin. The addition of six per cent of gelatin to milk reduces the curd tension to about one-half of its original value.

The above experiments show clearly that gelatin is very effective in reducing the curd tension of milk. But when these experiments were repeated with a sample of bulk gelatin from the hospital kitchen the reduction in the curd tension of milk was much smaller. In the literature the statement is occasionally found that gelatin has no effect on the curd tension of milk. This would indicate that all gelatins are not equally effective in reducing the curd tension of milk.

Riggs and Beaty (9) at a meeting of the American Chemical Society in New York City in 1935 reported that the lowering of the curd tension of milk by gelatin was related to the viscosity of the gelatin sample employed; i.e., the higher the viscosity of the gelatin the greater the reduction in curd tension.

We have been extremely fortunate in having placed at our disposal seven different preparations of gelatin,\* some properties of which are listed in Table 2.

The viscosity and jelly strength of these gelatin preparations were determined by the regular methods (10). The pH of a one per cent solution of the gelatin in water was estimated with the glass electrode.

Striking differences are noted in the properties of the porkskin and of

\* We are indebted to Dr. Thomas B. Downey for the data on the viscosity and jelly strength of the various gelatins used throughout this investigation.

TABLE 2

*The properties of some gelatin preparations used in experiments on curd tension of milk*

Number of gelatin preparation	Source of gelatin	Viscosity	Jelly strength	pH
		<i>millipoise</i>		
P-1 .....	Porkskin	65.0	305	3.82
P-2 .....	"	40.2	225	4.05
P-3 .....	"	28.7	139	4.20
P-4 .....	"	21.4	84	4.32
B-1 .....	Bone	51.6	246	6.08
B-2 .....	"	68.7	197	6.14
B-3 .....	"	71.0	166	5.90

the bone gelatin preparations. The porkskin gelatins show a much higher acidity than do the bone gelatins. In the case of porkskin gelatins, viscosity increases with increase in jelly strength, while for the bone gelatins, viscosity increases as the jelly strength decreases.

A study has been made of the effect of these gelatins on the curd tension of milk. A few changes have been introduced in the experimental procedure. The milk samples were obtained from Guernsey cows from a dairy farm about thirty miles from the laboratory. The samples were obtained at the afternoon milking and were kept at a low temperature until used the following morning. The milk was then brought to 25° C., 100 cc. were pipetted into the test bottles, which contained the weighed amount of gelatin, and the mixture was allowed to stand at room temperature for fifteen minutes. The test bottles were closed with a rubber stopper which was provided with a thermometer and a small outlet to allow for the expansion of air during heating. The jars were then transferred to a thermostat maintained at 40° C. and the contents were swirled around two or three times at five minute intervals. At the end of one-half hour the bottles were removed from the thermostat and allowed to stand, with occasional stirring, at room temperature until the temperature of the contents had dropped to 35° C. The curd tension was then determined either with the Hill apparatus or with the modified Bloom gelometer (2).

The results of two of a series of experiments are given in Figures 3-6. The curd tensions shown in Figures 3 and 5 were obtained with the modified Bloom gelometer for varying concentrations of the gelatins in milk, the original curd tension of which was 58 grams. Those given in Figures 4 and 6 were obtained in a similar experiment using the Hill apparatus and a different sample of milk, which also had an initial curd tension of 58 grams. Because of the differences in pH of the gelatin samples (to be discussed in detail below) the curves for the bone gelatin preparations and for the porkskin gelatin preparations have been drawn separately. The results show that within the limit of experimental error the reduction in curd tension

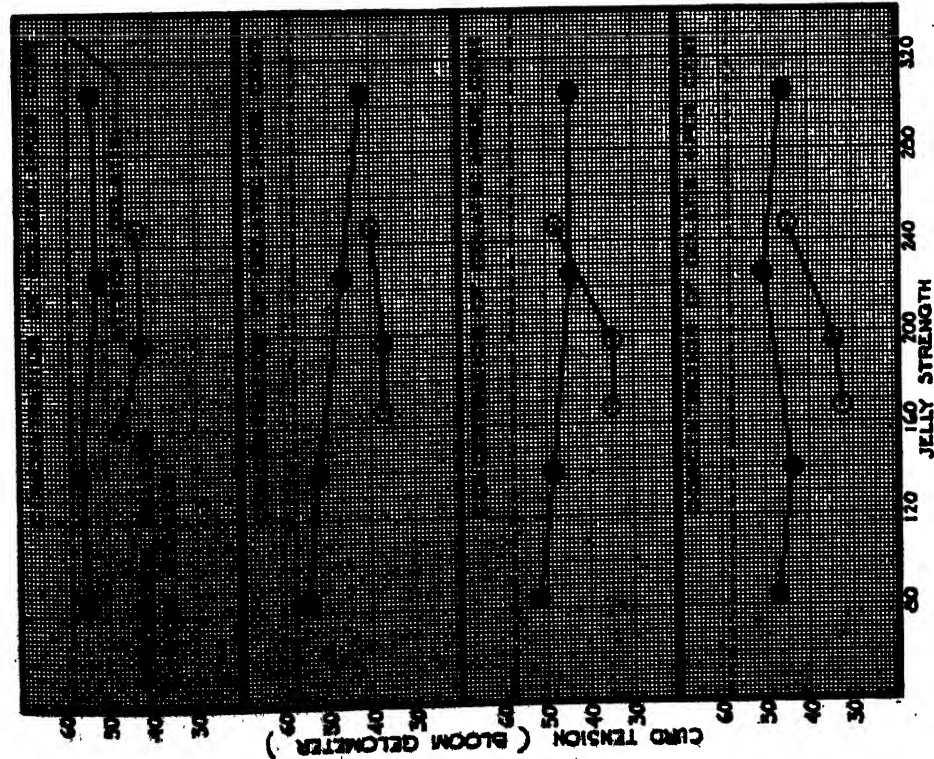


FIG. 4. Relation between curd tension and the viscosity of the gelatin.

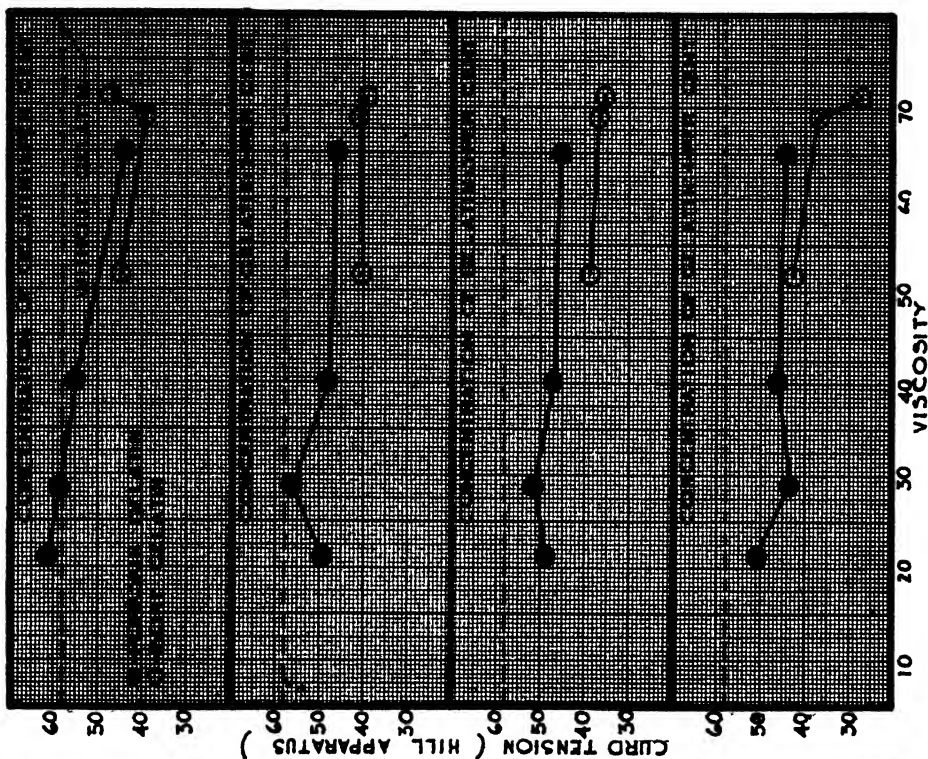


FIG. 5. Relation between curd tension and the jelly strength of the gelatin.

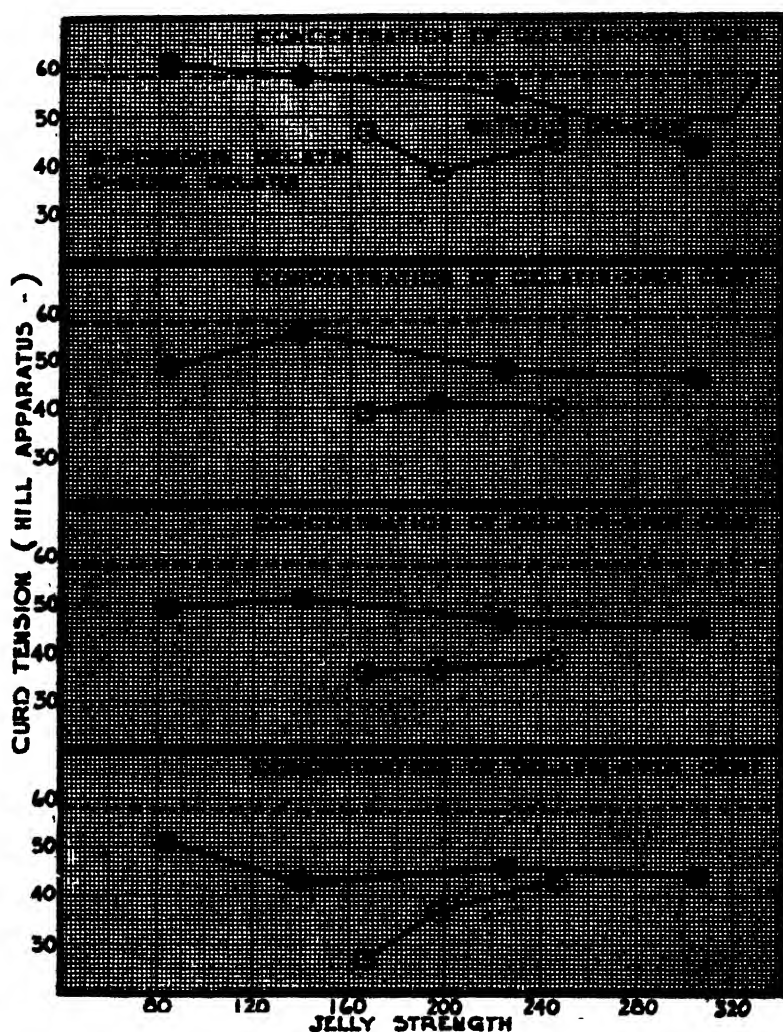


FIG. 6. Relation between curd tension and the jelly strength of the gelatin.

increases with increase in the viscosity of the gelatin preparation employed. If the reduction in curd tension is compared with the jelly strength of the gelatins, however, the two types of gelatin show a different behavior. With increase in jelly strength the reduction in curd tension increases in the case of the porkskin gelatins and decreases in the case of the bone gelatins. The most effective gelatin from the standpoint of reduction of curd tension was a bone gelatin with a high viscosity and a low jelly strength; the least effective, a porkskin gelatin with a low viscosity and a low jelly strength.

The percentage reduction in the curd tension of milk by the bone and by the porkskin gelatins is given in Table 3. The results are the average of six experiments on milks with initial curd tensions between 34 and 58 grams.

TABLE 3

*Percentage reduction in the curd tension of milk by various types of gelatin*

Gelatin number	Viscosity of gelatin	Average percentage reduction in the curd tension of milk by gelatins of the following concentrations			
		1 per cent	2 per cent	3 per cent	4 per cent
	<i>millipoise</i>				
P-4 . . . . .	21.4	6	14	17	21
P-3 . . . . .	28.7	5	12	21	28
P-2 . . . . .	40.2	10	18	29	25
P-1 . . . . .	65.0	17	22	28	24
B-1 . . . . .	51.6	21	33	31	30
B-2 . . . . .	68.7	30	37	44	41
B-3 . . . . .	71.0	24	39	44	48

In nearly every case an increase in the reduction of the curd tension follows an increase in the viscosity of the gelatin sample from the same source. However, if the gelatin samples are considered, irrespective of source, the P-1 gelatin is seen to occupy an anomalous position; *i.e.*, the reduction in curd tension is less than would be predicted from its viscosity. The extremely high jelly strength of the P-1 gelatin may explain this behavior, in part, at least; incipient gel formation during coagulation of the milk would tend to raise the curd tension reading and so lead to erroneous conclusions regarding the reduction in curd hardness. This effect would be greater the higher the concentration of gelatin in the milk.

These experiments explain why investigators working with one per cent solutions of gelatins similar in properties to P-3 or P-4 would fail to obtain a marked lowering of the curd tension of milk.

#### THE RELATION BETWEEN CURD TENSION AND THE CONCENTRATION OF HYDROGEN IONS IN MILK

In the early part of our investigation we studied the effect of the pH on the curd tension of milk alone and of milk to which had been added five per cent of gelatin (Knox Gelatine). Pasteurized milk was used in this work. A measured amount of 0.1400 N hydrochloric acid or of 0.1094 N sodium hydroxide was added in a fine stream from a burette to 100 cc. of milk. The mixture was rotated constantly during the addition to prevent any local excess of reagent. In the case of milk containing gelatin, the gelatin was first dissolved in the milk before the addition of the acid or the alkali. The pH was determined with the quinhydrone electrode. The curd tension was estimated by means of the Hill apparatus. The results are given in Figure 7.

The initial curd tensions of the milks varied from 17 to 43 grams. The curves represent approximately the average curd tensions of the milks at the various pH values. Nine complete titrations were performed on milk alone and six on milk with added gelatin. The pH was found to have a very marked effect on the curd tension. In the case of milk alone the average

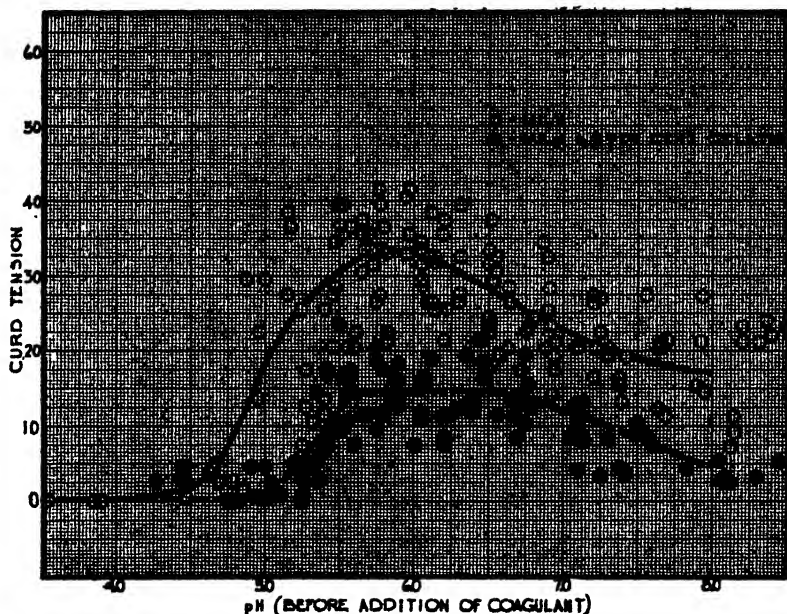


FIG. 7. Relation between curd tension and the pH of the milk.

curd tension curve reached a maximum at a pH of about 5.7 to 5.9. Beyond this point the decrease in curd tension with increasing acidity was probably due to the removal of the casein from colloidal solution by precoagulation with acid, which left less and less of the protein to react with the pepsin. The fall in curd tension on the alkaline side of the maximum was due, in part at least, to the inactivation of pepsin by the alkali. The average curd tension curve for the milk which contained five per cent of gelatin is striking in that here we do not have a maximum point, but rather a maximum zone which extends from approximately pH 5.6 to 6.6. Since the pH was determined with the quinhydrone electrode, no attempt was made to extend the curves beyond pH 8.

The addition of five per cent of the gelatin to the milk changed the pH on an average from 6.57 to 6.35. Such a pH change would in itself result in only a very slight increase in curd tension.

As has already been noted, a marked difference in acidity existed between the bone gelatin preparations and the porkskin gelatin preparations used in the present investigation. The effect of these gelatin preparations on the pH of the milk is shown below in Table 4. The pH values recorded here were estimated by means of the glass electrode.

Addition of two per cent of the various gelatins increased the acidity of the milk, the porkskin gelatins having a somewhat greater effect. After coagulation, however, the pH of the coagulated milk which contained two per cent of bone gelatin was the same as that of the coagulated milk to which

TABLE 4  
*The effect of various gelatins on the pH of milk*

Gelatin number	pH of original gelatin preparation	pH of a 2 per cent solution of gelatin in milk	
		Before addition of coagulant	After addition of coagulant
P-1 . . . . .	3.82	6.06	5.39
P-2 . . . . .	4.05	6.04	5.42
P-3 . . . . .	4.20	6.08	5.47
P-4 . . . . .	4.32	6.13	5.45
B-1 . . . . .	6.08	6.28	5.60
B-2 . . . . .	6.14	6.29	5.60
B-3 . . . . .	5.90	6.29	5.57

pH of original milk, before coagulation: 6.47  
 after coagulation: 5.60

no gelatin had been added, while the pH of the milk which contained the porkskin gelatin was slightly more acid. It is doubtful whether this change in acidity is large enough to cause any marked difference in curd tension; yet since slight differences do exist, it seems advisable at the present time to consider the two types of gelatin separately.

#### SUMMARY

In general, increase in the curd tension of milk was accompanied by an increase in casein concentration, although wide variations occurred. The relation of the curd tension to the concentration of the casein seemed to follow approximately an S-shaped curve.

The addition of gelatin (Knox) to milk caused a marked fall in curd tension. In most cases, two per cent of gelatin added to milks of curd tension up to about 50 grams converted them to soft curd milks.

The reduction in the curd tension of milk increased with increasing viscosity of the gelatin preparation employed. As the jelly strength of the gelatin preparation increased, the reduction in curd tension increased for the porkskin gelatins and decreased for the bone gelatins.

A bone gelatin with a high viscosity and a low jelly strength was most effective in lowering the curd tension of milk.

The pH was found to have considerable influence on the curd tension. The average curd tension of milk reached a maximum when the pH of the milk before coagulation was between 5.7 and 5.9. In the case of milk which contained five per cent of gelatin, the average maximum curd tension occurred when the pH before coagulation was between 5.6 and 6.6.

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# THE LIPASE, FATTY ACID AND CHOLESTEROL CONTENT OF COW'S BLOOD IN RELATION TO THE PRODUCTION OF RANCID MILK

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In a study of off-flavored milk (4) (5) (6), significant differences were found between the composition of normal and that of rancid milk produced by animals of the same breed while maintained under the same nutritional and environmental conditions. Rancid samples were lower in lactose and higher in chloride, fat, total solids, protein, titratable acidity and hydrogen-ion concentration than were normal samples obtained during same period of lactation. Of perhaps even greater significance, the lipolytic activity of rancid milk was found to be greater than that of normal milk.

The increased lipase content of rancid milk has come to be regarded as one of the causes, if not the sole cause of natural rancidity. The flavor of rancid milk is attributed to the presence of free fatty acids, an unusual amount of which is thought to be released as a result of the increased amount of lipase. It has been suggested, however, that other factors may contribute to the production of rancidity. There is the possibility of the presence in milk of some substance which acts either as an activator or an inhibitor of lipase activity and which may be present in larger or smaller amounts in rancid milk than in normal milk. Cholesterol suggests itself in this role; it is a normal constituent of milk and has been shown to exert an effect upon lipase activity. The exact nature of its influence, however, appears to be a matter of disagreement. Remezov and Tavaststyerna (7) consider cholesterol an important regulator of serum lipase activity. They found that *in vivo*, cholesterol inhibits blood lipase by adsorption. Later, however, Corran (2) reported that cholesterol in low concentration acts as an augments of lipolysis.

Inasmuch as blood is the ultimate source of milk constituents, it appeared possible that the blood of animals producing rancid milk might differ in certain respects from that of animals whose milk is normal. The increased lipase of rancid milk might be due to increased blood lipase; the free fatty acid giving rancid milk its flavor might be the result of elevated blood fatty acids; an unusual amount of blood cholesterol might bring about an unbalance between the cholesterol and lipase of milk, resulting in an abnormal lipolytic activity, with the consequent production of rancid milk. Determinations were therefore made of the lipase, fatty acid and cholesterol content of the

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blood of certain members of the herd under observation in a study of off-flavored milk. The present paper reports the results of these determinations.

#### EXPERIMENTAL

The cows used in the experiment were members of the college Jersey herd. The care and management of the herd have been described in detail in a previous paper (4). Milk samples obtained weekly from each member of the herd over a period of three years were scored for flavor and analyzed. Certain of these cows were selected as subjects of the blood study which was continued over a period of nine months. Blood samples were always taken early in the morning, approximately three hours after the cows were fed. The number of samples obtained from the individual animals varied, depending upon the length of time the animal was available for the experiment. In five cases practically complete lactations were covered.

Blood was drawn from the jugular vein into a centrifuge tube and the serum obtained by centrifugation after the clot had formed. The total lipids were determined by the oxidation method of Bloor (1). The fatty acids were calculated by difference after the determination of the cholesterol by the Liebermann-Burchard reaction, following Bloor's procedure. The lipolytic activity of the blood was determined by a procedure which was essentially the same as that employed by McGuire and Falk (3). This method determines the degree of hydrolysis of tributyrin effected by 15 ml. of a 1:1 dilution of serum during a 24-hour incubation period. The lipolytic activity of the serum is expressed as ml. of 0.1 N NaOH required to neutralize the acid released by hydrolysis.

The method used for estimating lipase activity was capable of detecting the presence of 0.5 cc. serum, as may be seen in Table 1 which shows the degree of hydrolysis produced by increasing amounts of serum.

TABLE 1  
*Degree of hydrolysis of tributyrin effected by increasing amounts of serum*

Ml. serum	Degree of hydrolysis ml. 0.1 N NaOH
0.5	0.6
1.0	1.0
5.0	3.3
7.5	4.6

The lipolytic activity of 112 blood samples was determined. Of these, 44 samples were taken on days when the animals produced rancid milk. The blood of animals producing rancid milk was found to effect the same degree of hydrolysis as did that of animals whose milk was normal. The mean titration for the former group was 4.48 ml., that of the latter, 4.41 ml. The

increased lipolytic activity of rancid milk may not, therefore, be explained on the basis of an increased lipid content of the blood.

Since few data are available on the fatty acid and cholesterol content of

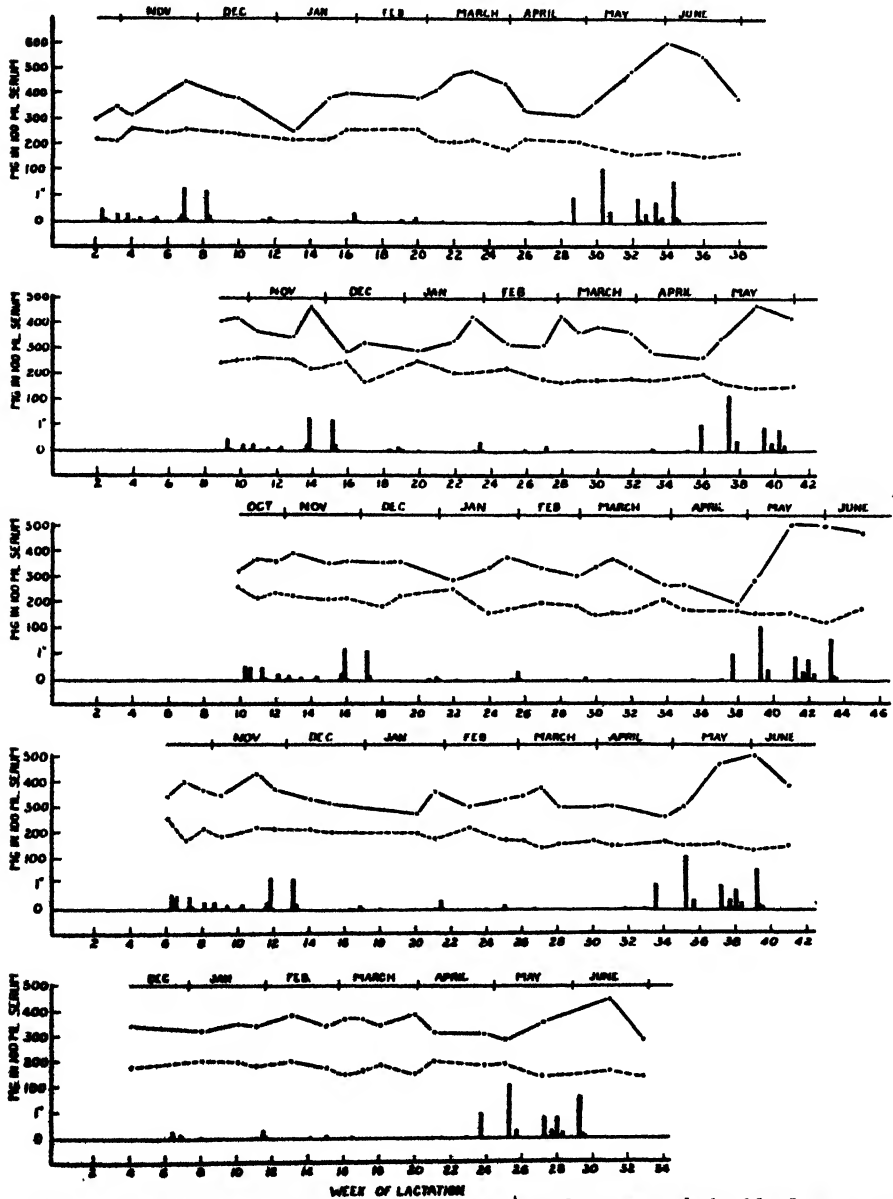


FIG. 1. Variation in the fatty acid and cholesterol content of the blood serum of individual cows during lactation.

———— Fatty acid.  
 - - - - - Cholesterol.  
 ——— Rainfall.

TABLE 2  
Influence of green pasture on the fatty acid and cholesterol content of the blood serum of cows

Animal number	Condition of pasture	Week of lactation	Fatty acids		Cholesterol	Animal number	Condition of pasture	Week of lactation	Fatty acids		Cholesterol
			mg. per 100 ml. serum	mg. per 100 ml. serum	mg. per 100 ml. serum				mg. per 100 ml. serum	mg. per 100 ml. serum	mg. per 100 ml. serum
1	D <sup>1</sup>	27	349		137	6	D	22	325	221	147
	G <sup>2</sup>	31	445		159		G	24	503	185	131
2	D	22	242		153	7	D	35	301	147	147
	G	26	388		112		G	39	506	131	131
3	D	17	259		138	8	D	17	315	242	242
	G	19	454		114		G	20	519	163	163
4	D	39	289		158	9	D	21	304	205	205
	G	41	498		171		G	24	507	144	144
5	D	32	498		171	10	D	22	299	213	213
	G	34	609		177		G	25	499	177	177

<sup>1</sup> Dry pasture.

<sup>2</sup> Green pasture.

the blood of lactating cows, individual curves for approximately complete lactations are shown for five cows in Figure 1. Two of these animals, numbers 4 and 5, frequently produced rancid milk. From the graphs it is evident that the trend of the cholesterol content of the blood paralleled that of the fatty acids but that changes in the fatty acids were the more pronounced. These constituents tended to increase during the first months of lactation then to decrease gradually as lactation advanced. The most marked variation occurred in the months of May and June when the fatty acids showed a pronounced increase without a corresponding change in the cholesterol. This marked rise in fatty acids was attributed to the improvement in the pasture, since, as may be seen in Figure 1, it occurred immediately after a period of rainfall which broke a long period of drought. All cows showed the rise in fatty acids, regardless of the period of lactation. This is shown in Table 2 which gives the stage of lactation and the fatty acid and cholesterol content of blood while the pasture was dry and after it became green.

Monthly variations in the fatty acids and cholesterol of the blood are shown in Figure 2. The graphs in this figure present mean values for all animals under observation. Values have not been included for those periods when the fatty acid content was increased because of green pasture. The

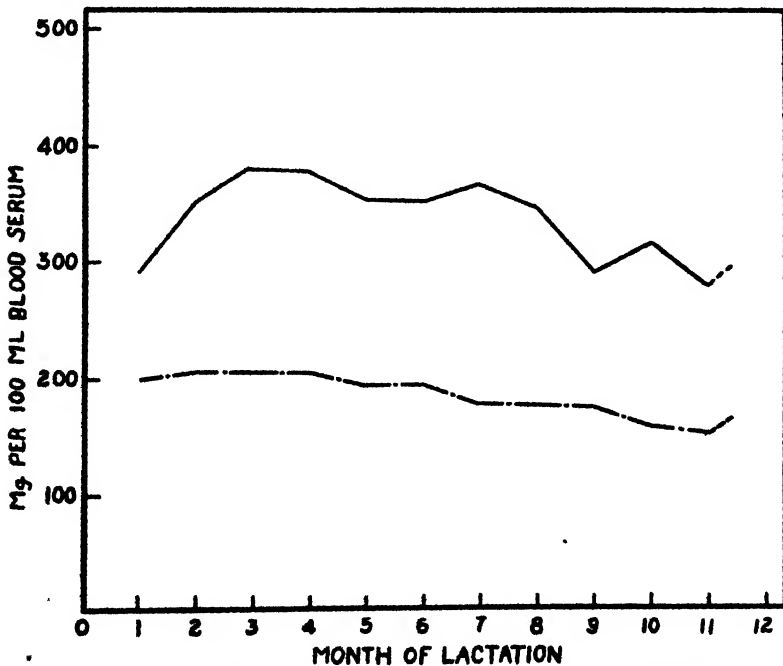


FIG. 2. The average fatty acid and cholesterol content of the blood serum of cows in relation to the period of lactation.

———— Fatty acid.  
 — . — . — Cholesterol.

fatty acid of blood was marked by a sharp rise during the first 12 weeks of lactation, a gradual decline during the succeeding 20 weeks, followed by a sharp decline which lasted until the end of lactation. The cholesterol content remained nearly constant for the first 24 weeks, then decreased gradually to the end of the lactation period.

A total of 248 blood samples were analyzed. In Table 3 are shown the mean fatty acid and cholesterol content of all samples taken on days when the milk was normal and the mean values for samples taken on days when rancid milk was produced.

TABLE 3  
*Mean fatty acid and cholesterol content of blood serum of cows producing normal and rancid milk*

Source of samples	Blood samples	Fatty acids	Cholesterol
	<i>number</i>	<i>mgs. per 100 ml. serum</i>	<i>mgs. per 100 ml. serum</i>
Cows producing normal milk .....	212	368.3	189.7
Cows producing rancid milk .....	36	359.7	213.7

It is evident from the table that there was no significant change in the fatty acid and cholesterol content of the blood of animals on days when rancid milk was produced.

During the period of the blood study seven cows produced rancid milk. Two of these animals had a blood fatty acid and cholesterol content higher than the average value for the same periods of lactation; the levels for the other five animals closely followed the normal. The mean fatty acid and cholesterol content of all samples of blood from the seven animals which produced rancid milk was 368 mg. and 192 mg. per 100 ml. serum, respectively, as compared with values of 388 mg. and 202 mg. for animals producing only normal milk. The differences are not significant.

#### CONCLUSIONS

Normal values for the fatty acid content of the blood serum of cows show a sharp increase during the first three months of lactation, followed by a gradual decrease which continues to the end of lactation. The cholesterol content of the blood shows a similar but less pronounced rise followed by a slight decline. A marked increase in blood fatty acids occurs when lactating cows are changed from dry to green pasture. This increase occurs regardless of the stage of lactation.

The fatty acid and cholesterol content of the blood serum of cows producing rancid milk follows the same trend as does that of cows producing normal milk during corresponding periods of lactation. There is no increased lipo-

lytic activity in the blood serum of cows producing rancid milk, although such milk has a greater lipase content than normal milk.

The production of rancid milk cannot be explained on the basis of a change in any one of the above blood constituents.

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# COFFEE AS A FACTOR IN THE FEATHERING OF CREAM

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The feathering of cream when mixed with hot coffee was first reported upon by Burgwald (1) in 1923. He found acidity of the cream and homogenization to be the most important factors causing this defect. The method of preparing the coffee, according to Burgwald, was not important as the hydrogen ion concentrations of the brew made by boiling, percolating and by the drip method were practically identical (4.91–4.92). Burgwald further stated that there was no difference in the effect of the various grades of coffee upon feathering. The method of combining the coffee and cream was of no great consequence although the cream feathered at a slightly lower acidity when the coffee was added to the cream and sugar mixture.

In 1930 Tracy and Ruehe (2) showed that the feathering of homogenized cream in coffee was closely related to the salt balance in the cream and coffee mixture. An excess of calcium or magnesium salts in either the cream or water used for making the coffee increased the tendency for the cream to feather. Cream high in acidity and that high in butter fat were found to be rather unstable. Lowering the fat content, reducing the acidity, increasing the serum solids, the addition of citrate salt, preheating and homogenizing at a high temperature, reducing the homogenizing pressure, and the use of two homogenizing valves instead of one, were factors found to reduce the tendency towards feathering through their effect in reducing fat clumping.

The work of Doan (3) substantiated the findings of Tracy and Ruehe. Whitaker (4) in studying the feathering of evaporated milk in coffee found that the milk was more likely to feather in strong than in weak coffee. Furthermore he found that the hydrogen ion concentration of the coffee remained constant and was independent of the method of preparation. Prolonged contact of the coffee and grounds, however, were shown to increase the quantity of soluble ash and consequently tended to increase feathering.

## PLAN OF STUDY

In order to obtain a better understanding as to what part coffee may play in the problem of cream feathering, samples of coffee were secured from a number of different processors and distributors. Altogether samples of twenty-six brands were obtained for this purpose. These coffees were compared from the standpoint of:—

1. Relative tendencies towards feathering.
2. Comparison of different methods of making coffee in relation to feathering.

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3. Significance of amount of coffee used.
4. Relation of coffee species, degree of roast and method of curing to feathering.
5. Extent to which age of the coffee affects its tendency to feather.
6. Procedure followed in mixing coffee and cream.

#### METHOD OF PROCEDURE

The degree of feathering was determined in coffee brew made from different water selected from the following group:

Water sample No.	Water used	Ave. pH
1	Tap	7.2
2	Distilled plus Mg. (10 ppm.) and Ca (30 ppm.)	6.95
3	Distilled plus Mg. (20 ppm.) and Ca (60 ppm.)	6.75
4	Distilled plus Mg. (30 ppm.) and Ca (80 ppm.)	6.70
5	Distilled plus Mg. (40 ppm.) and Ca (120 ppm.)	6.90

The coffee was measured with a measuring tablespoon and unless otherwise stated was used at the rate of one level tablespoon per measuring cup of water.

The regular procedure for making the coffee by the percolator method was to heat the coffee for five minutes after it began percolating. When made by the drip method the water was poured over the coffee in a regular coffee dripolator. When the coffee was boiled the percolating pot with the percolator removed was used.

The amount of feathering was determined using coffee brews at four different temperatures (120°, 145°, 170°, and 195° F.). One hundred ml. of coffee brew was poured into a 100 ml. glass graduate. When the temperature was properly adjusted 10 ml. of fresh 22% cream homogenized at 700 pounds pressure and at a temperature of 140° F. was added. The contents were mixed by pouring into a beaker and back into the graduate. The coagulum that rose to the top was then measured in terms of ml.

All pH measurements were made on the cold coffee brew, using a Coleman portable type apparatus with glass electrode.

The coffees were scored on the basis of the amount of coagulum formed during the feathering test. Although this method is to be criticized because of possible slight variations in the packing of the coagulum in the top of the graduate, the method is rapid and was thought to be sufficiently accurate to give relative values. Since the coffees made with water of varying calcium and magnesium content represented different degrees of severeness of the

test it was necessary to assign arbitrary values to these different coffees in order to arrive at a total score for each coffee made with the four different waters. The assumed values for each cubic centimeter of coagulum formed were as follows:

Temperature	Score value				
	Water 1	Water 2	Water 3	Water 4	Water 5
120° F. ....	*	*	*	*	*
145° F. ....	*	*	*	6	4
170° F. ....	*	*	5	3.5	2
195° F. ....	*	5	3	2	1

\* No value was assigned as feathering did not occur when using this water at the indicated temperature.

#### RELATIVE FEATHERING TENDENCIES OF COFFEES OF DIFFERENT BRANDS

To compare the relative feathering tendencies of coffees, samples of 26 different brands were used. The coffee brews were made by the percolator method. The results are given in Table 1.

Examination of the data in Table 1 will show some of the coffee brews to differ in their feathering tendencies. It was only when a water containing magnesium and calcium, added to the extent of 40 and 60 parts per million respectively, was used, that the brand of coffee became a factor. It may be concluded, therefore, that it would be only when unfavorable conditions existed that this tendency on the part of certain coffee to be a factor, would be of any significance. For example, when the cream was of high quality, contained a normal salt balance, and was not processed in such a way as to produce excessive fat clumping, and when the water used to make the coffee was not high in calcium or magnesium content, tendencies for certain brands of coffee to favor feathering more than other brands would be of no particular importance. However, there may be cases where the cream is so nearly destabilized when mixed with the hot coffee that the particular ingredient in the coffee responsible for the feathering may be just the factor necessary to bring about the coagulation. This condition may account for slight variations in results obtained by different dispensers of coffee brew that are being served by the same dairy.

Just what the coffee constituent is that favors cream feathering is not known. Whitaker (4) suggests the ash content of the bean as important in this respect. Acetic, formic and valeric acids have been reported by various investigators (5) of the composition of the volatile oils of coffee suggesting coffee acidity as a possible factor in feathering. While no exact correlation seems to exist between pH of the brew and feathering there are some general tendencies in this respect as shown by the data in Table 2. The lack of a clear cut correlation between the pH of the brew and feathering would indicate the presence of more than one constituent in the coffee that is related to the heat coagulation of the cream protein.

TABLE 1  
*Comparison of different coffees in their tendencies to produce feathering*

Brand No.	pH	Brew 1		Brew 2		pH	Brew 3		pH	Brew 5		Score*				
		ml curd formed		ml curd formed			ml curd formed			ml curd formed						
		145°	170° 195°	145°	170° 195°		145°	170° 195°		145°	170° 195°					
1	6.35	0	0	0	0	4.90	0	0	4.80	0	0	12	1.5	13.5	17	86
2	6.70	0	0	0	0	4.82	0	0	4.90	0	0	0	0	0	15	15
3	6.45	0	0	0	0	4.92	0	0	4.91	0	0	0	0	11	13.5	35.5
4	6.68	0	0	0	6	4.75	0	6	4.67	0	0	10.5	7.5	14	16	135.5
5	6.87	0	0	0	0	4.70	0	0	4.65	0	0	8	9	12.5	13.5	98.5
6	7.18	0	0	0	0	4.70	0	0	4.70	0	0	1	0	7	12	29
7	6.80	0	0	0	0	5.05	0	0	4.95	0	0	0.5	0	0	14	15.5
8	7.15	0	0	0	0	4.95	0	0	4.90	0	0	9	0	6	16	55
9	6.90	0	0	0	0	5.00	0	0	4.98	0	0	0	0	7	13.5	27.5
10	6.95	0	0	0	0	5.08	0	0	5.02	0	0	0	0	5	11.5	21.5
11	7.05	0	0	0	0	5.01	0	0	5.00	0	0	0	0	9.5	10	29
12	7.60	0	0	0	0	5.20	0	0	5.19	0	0	0	0	0	10	10
13	7.35	0	0	0	0	5.02	0	0	5.02	0	0	4	4	10	15	63
14	7.68	0	0	0	0	5.09	0	0	5.08	0	0	10	7	15	59	59
15	7.42	0	0	0	0	5.29	0	0	5.23	0	0	0	0	0	11	11
16	7.39	0	0	0	0	5.46	0	0	5.37	0	0	0	0	0	4	4
17	7.27	0	0	0	0	5.10	0	0	4.90	0	0	1	4	13	24	24
18	6.68	0	0	0	0	5.09	0	0	5.06	0	0	2	0	12	13	23
19	7.02	0	0	0	0	5.17	0	0	5.08	0	0	0	13	12	18	18
20	7.30	0	0	0	0	5.12	0	0	5.07	0	0	0	18	12	28	28
21	7.24	0	0	0	0	5.02	0	0	4.93	0	0	3	2	12	17	58
22	7.01	0	0	0	0	5.24	0	0	5.26	0	0	2	1	12	12	20
23	7.05	0	0	0	0	5.20	0	0	5.10	0	0	1	7.5	12.5	34.5	34.5
24	7.02	0	0	0	0	5.16	0	0	5.18	0	0	5	9	12	45	45
25	6.37	0	0	0	0	4.82	0	1	4.80	0	6	8	5	11	13	114
26	6.65	0	0	0	0*	5.16	0	0	5.10	0	0	0	0	11	11	114

\* The higher the score the greater the degree of feathering.

TABLE 2  
*Relation of pH of coffee brew to cream feathering*

Coffee No.	pH* Score**	
Group 1		
16	5.36 - 4	} Score 0-24 Ave. pH 5.08
12	5.15 - 10	
15	5.22 - 11	
2	4.83 - 15	
7	4.97 - 15.5	
19	5.09 - 18	
10	5.01 - 21.5	
22	5.20 - 22	
18	5.01 - 23	
17	4.97 - 24	
Group 2		
9	4.93 - 27.5	} Score 25-49 Ave. pH 4.97
20	5.05 - 28	
6	4.68 - 29	
11	4.99 - 29	
23	5.14 - 34.5	
3	4.88 - 35.5	
24	5.12 - 45	
Group 3		
8	4.91 - 55	} Score 50-74 Ave. pH 4.96
21	4.92 - 58	
14	5.05 - 59	
13	4.97 - 63	
Group 4		
1	4.80 - 86	} Score 75-150 Ave. pH 4.71
5	4.62 - 98.5	
25	4.77 - 114	
4	4.67 - 135.5	

\* Based on average of brews 2, 3 and 5.

\*\* Represents total score for groups 2, 3 and 5.

#### ORIGIN OF COFFEE AND METHOD OF ROASTING BEAN AS RELATED TO FEATHERING

There are two general methods (6) of curing coffee after the ripe pods or cherries have been picked from the trees. In countries such as Brazil which have a dry period during the harvest season they spread the ripe cherries out on drying floors to dry in the sun until the flesh which surrounds the coffee beans becomes a dry, shriveled shuck. The beans are then threshed free of the brittle shucks and after being screened for size and hand picked to remove imperfections are ready for market. This is known as the dry method of curing, as opposed to the wet method used in damper climate like that of Colombia.

In the wet method of curing the freshly picked cherries are first put through a machine which removes the greater part of the flesh. They are then placed in cistern-like reservoirs full of water where the remaining flesh is removed by controlled fermentation. This fermentation not only breaks

down the remaining flesh but also loosens a parchment-like skin that surrounds the beans. The beans are then artificially dried and screened.

The curing of the coffee has considerable influence upon the flavor characteristics of the bean. Brazil produces approximately two thirds of the world's supply of coffee, most of which is cured by the dry method, whereas Colombia, the next largest producer of coffee, due to its climatic conditions, uses the wet method.

Most of the better quality brands of coffee are for the most part blends of Santos and Colombian coffees. Bourbon Santos is a term used to describe the coffee which grows on the younger Santos type trees, a superior product to that grown on the older trees. Unwashed Salvador is a dry-cured coffee similar to the Bourbon Santos. The Maracaibo comes out of the port of Maracaibo, Venezuela.

Coffee is roasted to produce the desired flavor. Up to a certain optimum point the roasting eliminates the raw acrid taste of the green coffee and develops the true characteristic flavor.

To determine what relations there may be between the method of curing and the degree of roast of the bean to the feathering of cream, the tests recorded in Table 3 were made. The coffee was made by percolator method.

Although some differences were obtained in feathering tests using the different types of beans the data do not definitely indicate that the method of curing the bean has a relation to the occurrence of feathering. Unfortunately, it was not possible to secure samples of the same coffee cured by both the wet and dry methods. Such samples, if available, might make it possible to detect a minor difference that would not be evident otherwise.

In the case of the degree of roast, however, the data show rather clearly that the more roasting the bean is subjected to the less tendency there is for feathering to occur. The explanation for this effect is to be found in the higher pH value of the brews made from the beans receiving the most roasting.

TABLE 3

*Effect of method of curing bean and degree of roast upon pH\* of brew and extent of feathering*

Bean	Light roast		Medium roast		Dark roast	
	Score	pH	Score	pH	Score	pH
Bourbon Santos ..	114.25	4.86	88.5	4.90	84.5	5.00
Colombian (Washed) ..	139.00	4.84	123	4.92	101	4.97
Unwashed Salvador ..	97.00	4.91	81	4.95	63	4.97
		Light medium roast		Medium dark roast		
		Score	pH	Score	pH	
Washed Maracaibo .....		73.5	4.97	18	5.09	
Natural Maracaibo .....		35	5.01	0	5.13	

\* pH and score values represent the average values obtained with the coffee brews made with water Nos. 2, 4 and 5.

TABLE 4  
*Relation of amount of coffee used in brew to its tendency to feather*

Coffee No.	Amount coffee	pH	Brew 3				pH	Brew 4				pH	Brew 5				Ave. pH
			ml. curd formed					ml. curd formed					ml. curd formed				
			145°	170°	195°			145°	170°	195°			145°	170°	195°		
12	1 tb.	5.04	0	0	0	4.99	0	0	11	4.92	0	6	16	4.98			
	2 tb.	5.03	0	0	0	4.99	0	0	10	4.95	0	3	13	4.99			
	3 tb.	5.07	0	0	0	5.03	0	0	10	5.00	0	2	10	5.03			
15	1 tb.	5.02	0	0	4	5.00	0	2	11	4.91	1	12	14	4.98			
	2 tb.	5.01	0	0	0	4.95	0	0	10	4.89	0	8	13	4.95			
	3 tb.	4.98	0	0	3	4.94	0	3	10	4.91	0	10	13	4.94			
6	1 tb.	4.91	0	0	7	4.78	0	8	15	4.79	3	11	15	4.83			
	2 tb.	4.87	0	0	13	4.84	0	5	16	4.79	1	13	15	4.83			
	3 tb.	4.89	0	0	14	4.81	0	8	16	4.80	1	13	17	4.83			
9	1 tb.	4.81	0	0	4	4.79	0	2	11	4.72	0	4	14	4.77			
	2 tb.	4.79	0	0	9	4.74	0	3	12	4.73	0	9	14	4.75			
	3 tb.	4.82	0	0	6	4.79	0	8	12	4.77	0	8	14	4.79			

## METHOD OF MAKING THE COFFEE AS A FACTOR IN CREAM FEATHERING

Since methods of making coffee vary considerably with the ideas of the individual brewer, an attempt was made to determine to what extent such variations may be a factor in the cream feathering problem.

*Amount of Coffee Used*

The first variable studied was the amount of coffee used. Four coffees were used in this experiment, two that had been rated high from a feathering standpoint and two that had been rated rather low. The results of the feathering tests and the pH measurements of the brews are given in Table 4. It will be observed that there was no consistent variation in the effect of increasing the amount of coffee used in the brew upon the amount of curd formed, the tendency being towards uniformity in results. An explanation for these results is found in the pH measurements which show but slight differences in the brews containing varying amounts of coffee. Coffee brew undoubtedly contains a buffer substance in addition to the acid constituents which are present in more or less definite proportions regardless of the amount of coffee used.

*Method of Preparing Brew*

Coffee brews made from seven different coffees by three different methods—boil, percolator and dripolator—were compared for cream feathering tendencies, using water number 5. It will be noted from the data in Tables 5 and 7 that both pH and the extent of feathering varies but little with the method of preparing the brew although there is some indication that coffee made by the dripolator method will have a slightly lower pH and slightly greater feathering tendencies than that made by either the boil or percolator methods. Such differences may be attributed to variations in the extent to which certain volatile acids such as acetic are retained by the brew.

*Age of Coffee as a Factor*

If we are to believe the advertising propaganda of the coffee industry, coffees undergo certain chemical changes during storage that materially affect their quality. Though most investigations attribute the age deterioration of coffee to oxidation of the fatty constituents, Prescott (5) *et al.* are of the opinion that furfuryl alcohol plays an important part in the staleness of coffee. To determine to what extent age of the coffee may be a factor in cream feathering samples of varying age were secured from five different companies. It will be noted from the data in Table 6 that without exception the brews made from the older coffees had a lower pH and as would be expected the general tendency was for a greater feathering from these brews. It is also interesting to note that the coffee substitute responded in the same way as the coffees as far as pH and feathering were concerned. These data

TABLE 5  
*Relation of method of making coffee to degree of cream feathering*  
 (Water No. 5 used)

Coffee Brand No.	pH	Boil ml. curd formed		Score	pH	Percolator ml. curd formed		Score	pH	Dripolator ml. curd formed		Score
		195°	170° 145°			195°	170° 145°			195°	170° 145°	
4	4.72	15	9	49	4.70	15	9	53	4.72	15	10	68
11	4.90	12	6	24	4.90	8	9	26	4.87	14	7	28
10	4.95	12	10	32	4.91	9	7.5	24	4.89	13	9	31
10a	4.91	13.5	11	35.5	4.91	13	8.5	30	4.85	12	10	44
10b	4.85	13	12	49	4.82	14	11	56	4.85	16	6	48
10c	4.92	13	10	33	4.90	13	8	29	4.90	10	10	30
10d	4.94	13	0	13	4.95	7	2	11	4.97	13	0	13
Ave.	4.88			33.6	4.87			32.7				37.4

TABLE 6  
*Effect of age of coffee upon pH of brew and its tendency to cause feathering*

Coffee No.	pH		ml. of feathering						Score				
	Brew 3	Brew 5	195° F.		170° F.		145° F.						
			Brew 3	Brew 5	Brew 3	Brew 5	Brew 3	Brew 5					
4													
a. Fresh	4.80	4.76	14	16	10	13	0	8	166				
b. 2 years old	4.75	4.69	14	15	5	10	0	10	142				
Coffee substitute													
a. Fresh	4.85	4.84	5	16	1	12	0	0	60				
b. One year old	4.83	4.80	12	16	3	15	0	0	97				
10													
a. Fresh	5.00	4.91	0	9	0	7.5	0	0	24				
b. 5 months old	4.87	4.80	7	14	6	9	0	0	83				
11													
a. Fresh	4.95	4.90	6	8	0	9	0	0	44				
b. 5 months old	4.85	4.78	8	15	2	11.5	0	4	88				
1													
a. Fresh	4.80	4.78	13	15	0	11	0	0	76				
b. 6 months old	4.79	4.77	9	15	3	11	0	3	91				
c. 4 years old	4.73	4.70	14	15	7	11	0	6	138				

TABLE 7  
Comparison of the degree of feathering when adding cream to coffee or coffee to cream  
(Water V used)

Coffee number	pH	Cream to coffee ml curd formed			Score	Coffee to cream ml curd formed			Score
		145° F.				145° F.			
		195° F.	170° F.	145° F.		195° F.	170° F.	145° F.	
A—Percolator method									
4	4.70	15	9	5	53	15	12	12.5	88
11	4.90	8	9	0	26	9.5	12	0	33.5
10	4.91	9	7.5	0	24	9	10	0	29.0
a	4.91	13	8.5	0	30	13	9	0	31
b	4.82	14	11	5	56	12	11	3	46
c	4.90	13	8	0	29	13	9	0	31
d	4.95	7	2	0	11	6	5	0	16
Ave.					32.7				39.2
B—Boil method									
4	4.72	15	9	4	49	14	12	10	78
11	4.90	12	6	0	24	13	9	0	31
10	4.95	12	10	0	32	13	7	0	27
a	4.91	13.5	11	0	35.5	13	12	0	37
b	4.85	13	12	3	49	14	12	10	38
c	4.92	13	10	0	33	13	11	0	35
d	4.94	13	0	0	13	12	4	0	20
Ave.					33.6				38
C—Dripolator									
4	4.72	15	10	9	68	14	14	11	86
11	4.87	14	7	0	28	12	14	6	64
10	4.89	13	9	0	31	12	7	0	26
a	4.85	12	10	3	44	13.5	6	0	25.5
b	4.85	16	6	5	48	15.5	11	8	48
c	4.90	10	10	0	30	16	13	0	42
d	4.97	13	0	0	13	12	4	0	20
Ave.					37.4				44.4

indicate that certain chemical changes likely occur during the storage of the coffee that accentuates the beans' effect upon cream feathering. No attempt was made to determine the nature of this change.

### *Method of Combining Cream with the Coffee Brew*

The usual procedure in hotel and restaurant service is to add the cream to the coffee, although in some cases the reverse is true. That the former method might be expected to produce somewhat less feathering is indicated by the data in Table 7. Although the differences were not great a distinct trend will be noted toward greater feathering when the hot coffee was added to the cream. That the significant factor is the rapidity with which the coffee and cream mix when combined is illustrated by the fact that creams which ordinarily do not feather may be made to do so by slowly adding the cream to the coffee so that it more or less floats on the surface. This is particularly true when the quantity of cream added is less than ordinary.

### CONCLUSIONS

In comparing the feathering tendencies of the brew made from 26 different brands of coffee the following conclusions are drawn:

1. Some coffees are more likely to cause feathering than others. This difference is likely due to variations in the pH of the brews resulting from variations in the soluble acids present in the beans.
2. It could not be shown that the method of curing the beans has any relation to the pH of the brew and feathering.
3. The degree of roast to which the bean is subjected affects feathering. The more roasting the bean is subjected to the higher the pH of the brew and the less the degree of feathering.
4. The concentration of the coffee in the brew has no definite relation to feathering.
5. The method of brewing, i.e., boil, percolator or dripolator, is not of great importance although slightly more feathering was obtained with brews made by the dripolator method.
6. The brew made from aged coffee has a lower pH and is more likely to produce feathering than that made from fresh stock.
7. More feathering may result when the coffee is added to the cream than when the cream is added to the coffee. Rapidity of mixing is thought to be the limiting factor.

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# THE THIRTY-THIRD ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. STOLTZ

*Secretary-Treasurer*

The American Dairy Science Association was called to order by the President, H. W. Gregory, in Campbell Hall on the campus of the Ohio State University on Tuesday morning, June 14, 1938, at 10:30 A. M., for the thirty-third annual meeting.

The program printed in the May issue of the Journal of Dairy Science was arranged by a program committee headed by Dr. T. S. Sutton. The May issue of the Journal also contained the abstracts of the various papers presented.

Vice-President Lewis L. Morrill, Ohio State University, gave an address of welcome. President H. W. Gregory gave the following response:

## PRESIDENT'S ADDRESS

"Today our Association meets for the thirty-third annual meeting and it is interesting to note that we have among our membership a number of men, who laid the early foundation for the American Dairy Science Association, still taking a very active part in the Association.

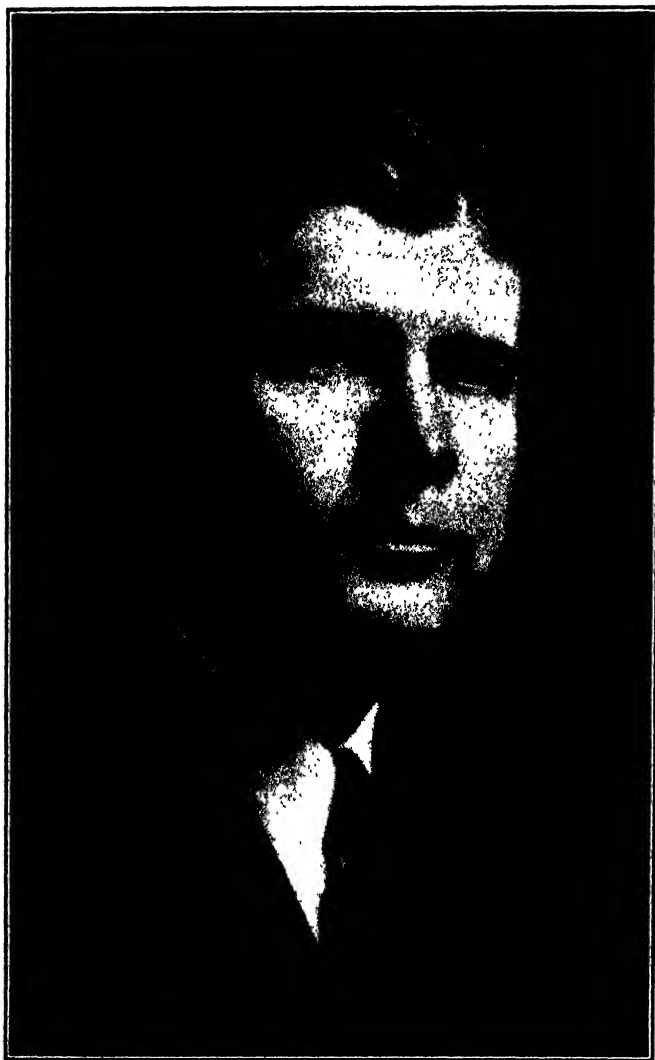
In 1906, when the American Dairy Science Association was first started under the name of "The Official Dairy Instructors' Association," our problems were somewhat different from those we have before us today. The name of our Association in the beginning was very descriptive of one of our early problems which was "what to teach and how to teach it." If we read available dairy reports, catalogue descriptions of courses offered in dairying, note equipment used in our dairy laboratories in our agricultural colleges at the time our Association started, and compare the dairy curricula and facilities for research at the present time in our schools, we realize the development that has taken place during this comparatively short period in the dairy industry. This Association has played a very important part in bringing about these changes. Our industry has been and is being revolutionized by science. We who are connected with the dairy industry are constantly called upon to readjust our thinking and methods of teaching as progress is made in dairy research. Due largely to many new developments in our industry and improved methods of teaching, a number of our members have expressed an interest in having a committee of this Association study the dairy curricula offered in our different institutions. A rather comprehensive committee was appointed rather late in the year for this purpose and a complete report cannot be expected this year, but with the enthusiasm and interest of Dr. H. B.

Ellenberger, general chairman of this committee, I am sure we can look forward to a later report which will be of considerable interest to a large number of our members.

Our organization has always given a great deal of attention to problems relating to inspection, sanitation, standardization of equipment, methods and products. In 1911, as a result of the activities of a committee from this Association, the first draft of standardizing testing glassware was made, and later a number of states passed what is generally called a milk and cream testers' license law, requiring the use of standardized glassware. A resolution made by the committee on sanitary procedure of the International Association of Milk Dealers was sent to this Association during the year, calling attention to the need of cooperation in problems having to do with sanitation. This resolution recommended that a program be undertaken cooperatively to determine good industry practices by survey and by original research by national dairy organizations interested. Mr. Ernest Kelly, Assistant Chief of the Bureau of Dairy Industry, United States Department of Agriculture, was appointed chairman of the committee to represent the American Dairy Science Association on this important project.

During the early history of our Association we gave considerable attention to dairy laws, rules, and regulations, especially those enforced by our state and city governments. Due to improvement in our methods of production, processing and improvement in transportation facilities for milk and cream, a large amount of these products today enters into interstate commerce. Many of the states have milk and cream testers' license laws, state cream grading laws, and state laws relating to methods and equipment used in production. There is very little uniformity in the different state dairy laws and if there were the enforcement in the different states would not likely be uniform. But to this increased interstate traffic of milk and cream, are we not in need of a federal law which would give some federal agency jurisdiction in such shipments of milk and cream regarding the weighing, testing, and grading, especially when cream or milk originates in a state which enforces such state laws? A federal agency having jurisdiction over interstate shipments would tend to bring about uniformity of state dairy laws, would be a great aid to states now enforcing testing, grading and sanitary laws, and would be an encouragement to the dairy industry in those states that do not have such laws to propose similar laws to their legislatures.

As our Association increases in membership and importance the demand for the American Dairy Science Association to approve or condemn certain proceedings, methods or statements is increasing, and while the policy of this Association has been very conservative along this line, there has not been any definite procedure to follow in securing approval of this



**EARL WEAVER, PRESIDENT**

Association for such requests. Last year a committee was appointed at our annual meeting to give this consideration and suggest a procedure to be followed when such requests are submitted to the Association or any section of the Association. This committee has made its report which will be considered by the directors, and I hope that from this report the Association will be able to adopt a definite policy in handling such requests in the future.

There has been considerable interest among the membership in forming junior chapters of the American Dairy Science Association. Prof. J. A. Nelson, member of the Board of Directors, has attempted to make a survey this year to find out how general is the demand for such an organization, and also to determine what degree of success similar organizations have had in forming such chapters. If the Board of Directors thinks that there is enough interest in junior or local chapters of the Association, after considering Professor Nelson's report, recommendation for adopting such a procedure will be made to the Association.

Last February I received a letter from Dr. A. C. Dahlberg, editor of our Journal, stating that he did not wish to be reappointed as editor of the JOURNAL OF DAIRY SCIENCE. During the ten years Doctor Dahlberg has been editor our Journal has continued to increase in importance and recognition in the scientific field. Research articles in the Journal have increased over 50 per cent. The Journal was changed from six to twelve issues yearly. Abstracts of literature on dairy cattle and literature reviewing articles on timely, selected topics are only a few of the many services added to the Journal in the last few years. No one thing indicates the sound basis on which the Journal has been built better than the fact that all these services have been added without increasing the annual dues. These extra services have demanded more and more time of the editor in reading manuscripts, galley proofs, indexing of articles, and increased correspondence until it is nearly impossible to handle the Journal incidental to one's work. Doctor Dahlberg stated that it is with a real sense of personal loss and regret that he asked to be relieved of the position of editor.

A meeting of the Journal Management Committee, Doctor Dahlberg and myself, was held in Chicago, March 5, 1938, at which time the position of editor for the Journal was gone over very thoroughly with Doctor Dahlberg with the hope that some arrangement might be made so that he would continue as editor. Upon the insistence of Doctor Dahlberg, that his resignation be accepted and a new editor be secured, the Journal Management Committee was asked to give consideration to the securing of an editor for the JOURNAL OF DAIRY SCIENCE and to make recommendations in time to submit to the Board of Directors at the annual meeting in June.

Doctor Dahlberg has been a very capable editor. He has spared neither time nor effort to make the Journal a success, and our Association greatly appreciates the splendid services he has rendered the Association as editor and regrets that he considers it necessary to resign at this time.

Our Association has been growing rapidly and a great deal of time in the last few years has been given to improving our organization and getting it on a good operating basis. There is no better evidence of the success of our efforts in this direction than the secretary-treasurer's report for the year ending December 31, 1937, which shows our total assets now amount to a little over \$17,000, \$9,000 of which is invested in government bonds. The public accountant, who was secured to audit our books for 1937, makes the following statement in his report: "The comparative profit and loss statement reveals the changes in this year's business as compared to the two previous years. The income of 1937 exceeded the income of 1935 by \$4,610.38. Although the increase over 1936 was only \$1,305.28, it is interesting to note that there was a switch from associate subscribers to members and subscribers. Advertising increased \$992.93, while operating expenses for the period increased only \$296.78. These results indicate that the Association is arriving at the point of economical operation when the profits were increased \$1,305.38 over the previous year with an increase in expense of only 22.7 per cent."

Our secretary-treasurer, Prof. R. B. Stoltz, deserves a great deal of credit for the interest, time and effort he has devoted to the American Dairy Science Association and the business management of our Journal. At the present time we have 1037 members and 825 subscribers, and our secretary hopes that by the end of the year the number receiving the Journal will increase to at least 2000. To keep our Journal on a sound financial basis and make the improvements that are desired, membership and subscriptions are very necessary. Due to the fact that our membership is widely scattered, the maintaining or increasing of our membership in this association must be done largely by individual members accepting some responsibility in securing members or subscribers. In January, this year, a representative in each state where we have members or subscribers was appointed with the hope that these representatives would make a special effort to see that those who are eligible would have an opportunity to subscribe to our Journal. Due to the rapid development and changes in our industry, there are a number of problems which many of our members believe should have the attention of our Association, but because of lack of time, finances, etc., we have not been able to give all these problems the consideration they deserve.

At this time I wish to express my personal appreciation to the numerous individuals, committees and officers of this Association for their assistance and cooperation during the year, and, especially, I wish to

commend Dr. T. S. Sutton and his committee for getting together our annual program in time to have it published and in the hands of our members several weeks before the annual meeting. This policy followed by our program committee has already added a great deal to our meeting and evidence of the value of such a procedure will undoubtedly be more in evidence before our meeting closes.

#### GUEST SPEAKER

Dr. K. Hickman, of the Eastman Kodak Company, Rochester, New York, was then introduced and gave a most interesting and instructive talk on "Vacuum Extraction of Accessory Food Factors."

#### ATTENDANCE

According to J. H. Erb, who had charge of the registration, the attendance showed that there were 620 registered, which is the largest registration at any of our annual meetings. Thirty-nine states, the District of Columbia, Canada and two foreign countries were represented. There were 358 members, 89 non-members, 125 ladies and 48 children registered.

S. M. Salisbury, chairman of the committee to submit new by-laws, presented mimeographed copies of the by-laws to each person present, and it was announced that these by-laws would be discussed and voted upon at the business session Thursday at 3:30.

After lunch the program as outlined was followed.

Tuesday evening the President of the University and the Dean of the College of Agriculture gave a reception for the Association at the Faculty Club.

Wednesday, June 15, the papers were read as provided for in the program. The men were given a complimentary barbecue lunch in the judging pavilion. In the evening the visitors were entertained in University Hall by a "rube" band and colored movie films of a trip around the world. They then were entertained in the natatorium by a diving and swimming exhibition. Following the evening entertainment a social time was then had between the natatorium and gymnasium.

On Thursday, June 16, papers were read and the business session was provided for as in the program. Thursday evening the annual banquet was given at the Neil House.

The ladies and children were entertained as provided for in the program published in the May Journal.

## GENERAL BUSINESS MEETING

## AMERICAN DAIRY SCIENCE ASSOCIATION

COLUMBUS, OHIO, JUNE 16, 1938

President Gregory called the meeting to order at 3:30 P. M., in the auditorium of Campbell Hall, Ohio State University, on Thursday, June 16, 1938. One hundred twenty-seven members were present.

The secretary-treasurer read the financial report which had previously been approved by the Board of Directors. Upon motion duly seconded the report was accepted and referred to the Auditing Committee.

Herbert Otting, chairman of the Auditing Committee, then gave the following report:

Columbus, Ohio  
April 13, 1938

To the Members of the  
American Dairy Science Assn.:  
Gentlemen:

The Auditing Committee of the American Dairy Science Association has made an examination of the books and statements of the Secretary-Treasurer as of December 31, 1937.

It is our opinion, based upon such examination, that the books have been kept accurately and that the balance sheet and related summary of profit and loss fairly present the financial condition of the American Dairy Science Association.

Respectfully submitted,

T. S. SUTTON  
W. L. SLATTER  
H. E. OTTING, *Chairman*

## CIRCULATION OF JOURNAL

The Secretary then submitted a map of the United States showing the circulation of the Journal in each state and the allotted number which was obtained by dividing the population for each by 50,000 persons. The District of Columbia had the highest circulation, and Vermont was the highest state having a circulation of 21 and an allotment of 7. The states leading in total circulation are as follows: Ohio, 157; New York, 154; Illinois, 114; Pennsylvania, 94; California, 90; Massachusetts and Wisconsin, 56; Minnesota, 50; Michigan and District of Columbia, 49. At the present time the total circulation is 1898 of which 1073 are members, 704 subscribers and 121 associate subscribers. At the annual meeting a year ago the total circulation was 1741, or 157 less than today.

Thus far this year we have obtained 215 new members. These new members are from the following states: Ohio, 65; New York, 15; Pennsylvania, 14; Massachusetts, 12; Maryland, 10; California, 9; Michigan and Indiana, 8; Vermont, 6; Washington, Utah and Illinois, 5; Canada, Wis-

consin, New Jersey, Minnesota, Iowa, Dist. of Columbia and Connecticut, 4; Missouri and Tennessee, 3; and 19 scattered.

The problem of membership is that we have such a large number of members that lapse each year. If the students graduating from the various dairy schools were educated to the idea that in order to keep up with the times and prevent themselves from falling in a rut, it is essential that they affiliate themselves with this Association, you would be doing your students a great favor and the problem of recruiting new members would be solved.

#### REPRINTS FOR BULLETINS

Many experiment stations and colleges are now using reprints from the JOURNAL OF DAIRY SCIENCE instead of having technical bulletins printed. It is much less expensive for you to buy reprints from the JOURNAL OF DAIRY SCIENCE after having had an article accepted, than it is to print your own bulletins.

#### MORE ACTIVITY IN THE PRODUCTION FIELD

The Association is now printing abstracts in the field of dairy production. An effort should be made to obtain advertising from the breed associations, from companies manufacturing dairy feeds and other equipment for dairy cows. An effort should also be made to increase our membership among commercial men in the field of production.

Early this year the wife of a college graduate presented her husband with a membership in the Association for his wedding anniversary gift. With such a helpmate, he will not be permitted to fall in the rut.

#### PRODUCTION SECTION

Mr. W. E. Krauss, chairman of the Production Section, read the following report:

The Production section held five sessions at the regularly scheduled hours and places with the Section chairman, W. E. Krauss in the chair for the Symposium on Nutrition; H. W. Cave, chairman for the pasture, hay and silage session; W. E. Krauss, chairman for the vitamin and mineral session, the milk secretion, metabolism and udder disease session; and J. B. Fitch, chairman for the final session on Thursday afternoon.

All sessions were very well attended, at times almost taxing the seating capacity of 200 in Room 100 of the Botany and Zoology Building, where the sessions were held. The papers presented were without exception well prepared, well presented and in most cases accompanied by slides, mimeographed charts and tables, or other illustrated material. The availability of the printed abstracts in the hands of the members was commented upon by many as being a material help in following the presentation of the wide range of subjects reported upon.

All but two of the papers listed on the printed program were presented. The marked program attached indicates the author making the actual presentation.

The business meeting of the Production Section was called at 4:15 on Wednesday afternoon. The minutes of the 1937 meeting at Lincoln, Nebraska, were read and approved.

Reports were submitted by the various standing committees and approved at the time, or in a short additional business meeting held at 11:30 on Thursday morning. Copies of these reports are attached.

Points of particular interest incorporated in the reports follow:

Breeds Relations Committee, C. N. Shepardson, chairman.

1. The cow year method of calculating herd averages was approved.
2. The feeding or injection of thyroxine to cows on official test was considered an undesirable and unacceptable practice.
3. It was suggested that no changes be made in the uniform herd test blank.

Committee on Rules for Conduct of the Students National Dairy Cattle Judging Contest, I. W. Rupel, chairman.

It was recommended:

1. To use numbers instead of letters in designating animals.
2. To change the score card for grading oral reasons (copy attached).

Committee on Awards for the Students National Judging Contest, A. A. Borland, chairman.

It was recommended:

To attempt to increase the number of scholarships available for winners in the Students National Judging Contest.

Committee on Methods of Measuring Results of Pasture Investigations, R. N. Lush, chairman (presented by G. Bohstedt).

Two items were emphasized:

1. Conditions for collecting samples, especially for mineral analysis, were enumerated.
2. A study of the size and number of clip plots is to receive continued attention.

Committee on Standard Methods, A. E. Perkins, chairman.

It was recommended:

That the committee be increased to include a specialist in the following fields:

- a. Milk analysis
- b. Blood and urine analysis
- c. Feeds and feces
- d. Endocrines
- e. Vitamins
- f. Enzymes

The chairman of the Production Section will appoint additional members to this committee after due consideration.

All standing committees were reappointed with their present personnel except for the Breeds Relations Committee. E. E. Heizer and W. L. Crandall were appointed for a period of three years to replace C. N. Shepardon and E. N. Schultz whose terms expired. H. A. Herman was appointed for a period of two years to replace Earl Weaver who resigned.

A. C. Ragsdale, chairman of the Nominating Committee, presented names for the offices of vice-chairman and secretary for 1939. A. H. Kuhlman of Oklahoma was elected vice-chairman, and A. L. Beam of Pennsylvania was elected Secretary. H. W. Cave of Kansas, vice-chairman for this year, automatically becomes chairman for 1939.

Respectfully submitted,

W. E. KRAUSS, *Chairman*

I. R. JONES, *Secretary*.

Upon motion duly seconded the report was accepted.

#### EXTENSION SECTION

E. N. Shultz, chairman of the Extension Section, submitted the following report:

The proceedings of the annual meeting of the Extension Section of the American Dairy Science Association were held June 14, 15 and 16, 1938, in the Horticulture Building of the Ohio State University at Columbus, Ohio, with Earl N. Shultz, chairman of the Section, presiding.

The program was organized and conducted under the direction of a program committee consisting of C. L. Blackman, Ohio State University; R. G. Connelly, Virginia Polytechnic Institute, and S. J. Brownell, Cornell University.

Twenty papers were presented and discussed during the sessions with an average attendance of eighty-six people. An instructive exhibition of extension educational methods and results was organized and presented by the Exhibits Committee composed of E. C. Scheidenhelm, Michigan State College; A. J. Cramer, University of Wisconsin; Floyd Arnold, Iowa State College; Leland Lamb, Cornell University. The states that furnished exhibits were: Massachusetts, New York, Connecticut, Virginia, Tennessee, National Dairy Council, Michigan, Vermont, Wisconsin, Kansas and Iowa. The field application of each exhibit was discussed before an audience of forty-five extension dairymen and others.

The general program committee was ably assisted by collaborating committees on Extension Exhibits, Dairy Cattle Breeding, Dairy Cattle Feeding, Production Testing, 4-H Dairy Clubs, Dairy Quality Improvement, Resolutions and Nominations. The chairmen of the committees presided for the presentation of their respective committee papers. The per-

sonnel of the committees, the title of papers and subjects and the schedules followed are indicated in the official American Dairy Science Association program.

The annual business meeting of the Extension Section was held Wednesday, June 15, 1938, at 4:00 P. M., in the Ohio State University Horticulture Building. At this meeting the following resolutions were adopted.

1. Whereas the officers and the Extension Section Program Committee, C. L. Blackman, Ohio State University; R. G. Connelly, Virginia Polytechnic Institute, and S. J. Brownell, Cornell University, contributed much time and thought to planning and developing this year's program, and as a result of their efforts a constructive program was evolved; therefore, be it resolved that we commend these officers and the program committee, and also the chairmen and members of the collaborating committees, for their fine accomplishments, and thank those who prepared papers and furnished exhibits. Furthermore, be it resolved that the extension exhibits be continued next year with a wider participation by the States.

2. The members of the Extension Section wish to thank the members of the faculty of the Animal Husbandry, the Dairy Production, and Dairy Technology Departments of the Ohio State University for the fine facilities placed at our disposal and the many courtesies extended to us, both of which have helped to make our meetings successful and enjoyable; therefore, be it resolved that a copy of this resolution be sent to Professors C. W. Gay, R. B. Stoltz and S. M. Salisbury and the Ohio Agricultural Experiment Station.

3. Whereas uniform rules and regulations for conducting Dairy Herd Improvement Associations were adopted by the Extension Section of the American Dairy Science Association one year ago and published and widely distributed by the United States Bureau of Dairy Industry, and whereas these regulations have been adopted in the main by most of the States; and whereas this Committee believes that the complete adoption of such rules would strengthen confidence in the Dairy Herd Improvement Association work throughout the country; therefore, be it resolved that the Extension Section urge all the States to put these regulations into effect.

4. Whereas the identification and permanent herd record program is now well underway with satisfactory results, and whereas the system was evolved after much effort, discussion and trial; therefore, be it resolved that we express our appreciation to the Bureau of Dairy Industry in general and to Dr. J. F. Kendrick, Chief, Division of Dairy Herd Improvement Association Investigations, in particular for the prompt and complete manner in which the records have been returned to the States.

A new secretary is elected for the Section each year and the other

officers are promoted, with the retirement of the incumbent president. O. J. Hill, Washington State College, was elected secretary, R. G. Connelly, Virginia Polytechnic Institute, succeeded to the office of vice-chairman and S. J. Brownell, Cornell University, became chairman of the Extension Section for 1939.

EARL N. SHULTZ

R. G. CONNELLY, *Secretary*

#### MANUFACTURING SECTION

B. E. Horrall, secretary of the Manufacturing Section, submitted the following report:

The Manufacturing Section held its meetings at the place and time indicated in the official program. All of the papers listed were presented with the exception of M-28, M-31 and M-37. The papers were very instructive and all of the sessions were well attended.

A motion was made to elect a vice-chairman in addition to a chairman and secretary this year. For succeeding years only a vice-chairman, who would automatically become chairman the forthcoming year, and a secretary would be elected. The motion was seconded and carried.

Reports were heard from the following committees:

1. Chemical Methods for the Analysis of Milk and Dairy Products
2. Quality Program for Dairy Products
3. Judging Dairy Products
4. Methods of Determining the Curd Tension of Milk
5. Score Cards for Sanitary Inspection of Dairy Farms and Milk Plants
6. Methods for Measuring the Oxidation of Milk Fat
7. Methods for the Bacteriological Analysis of Milk and Dairy Products. A financial report was also given for the sale of Bacteriological reports.

P. A. Downs of Nebraska was elected chairman; F. H. Herzer of Mississippi, vice-chairman; and J. I. Keith of Oklahoma, secretary, for the forthcoming year.

C. J. BABCOCK, *Chairman*

B. E. HORRALL, *Secretary*

#### COMMITTEE REPORTS

T. S. Sutton, chairman of the Program Committee, submitted the report for the committee as read in the minutes of the Board of Directors. Upon motion duly seconded the report was adopted.

In the absence of S. M. Salisbury, the by-laws, as read in the minutes of the Board of Directors, were presented by Secretary Stoltz, and upon motion duly seconded they were adopted.

PROPOSED BY-LAWS OF THE AMERICAN DAIRY SCIENCE  
ASSOCIATION

## ARTICLE I—MEMBERSHIP

Section 1. Any person is eligible to membership who is formally announced by an Agricultural College or Experiment Station, or by the Bureau of Dairy Industry of the United States Department of Agriculture or by the Canadian Department of Agriculture as an instructor, extension worker, investigator, or administrative officer connected with the dairy industry, or any person filling a position of responsibility connected with the dairy industry who has had a college or university training in technical science, or any person filling a responsible position in the dairy industry of a professional character requiring a technical knowledge of dairying of a high order.

Section 2. Nominations for membership shall be submitted to the Secretary-Treasurer in writing signed by the applicant and endorsed by at least one member. In case of uncertainty regarding the eligibility of the applicant for membership, the Secretary-Treasurer shall refer the application to the Board of Directors for decision. Upon receiving the approval of the Secretary-Treasurer, or the Board of Directors, when the application has been referred to them for action, and the payment of the membership fee and dues, the applicant shall be enrolled as a member of the Association.

Section 3. The membership fee shall be set by the Board of Directors and shall be payable with the application for membership.

Section 4. The annual dues shall be set by the Board of Directors and shall be payable on or before January first of each year.

Section 5. Any member of the association in arrears for dues for more than one year shall cease to be a member of the association but may be restored without the formality of re-election by payment of all arrears including the current dues.

## ARTICLE II—OFFICERS

Section 1. The officers of the Association shall be President, Vice-President, Secretary-Treasurer, Journal Editor and a Board of Directors.

The Vice-President shall be elected by the vote of the membership and his term of office shall be for one year beginning October first following his election. On the completion of his term of office as Vice-President he shall automatically become President for one year, or until his successor is duly chosen. The Secretary-Treasurer and the Journal Editor shall be elected by the Board of Directors for such term of office as the Board of Directors shall prescribe.

Section 2. The Board of Directors shall consist of ten members: six to be elected by the membership, the retiring President, the President, the

Vice-President, and the Secretary-Treasurer. The Secretary-Treasurer shall be an ex-officio member.

Two Directors shall be elected each year, whose terms of office shall be for three years. The terms of all Directors shall begin October 1, following election.

Section 3. The Board of Directors shall elect two members from the Association, who, with the Secretary-Treasurer as an ex-officio member, shall constitute the Journal Management Committee, which shall be responsible to the Board of Directors. The term of service of the elected members shall be at the discretion of the Board of Directors.

Section 4. The Board of Directors may constitute and appoint such committees not provided for in the By-Laws of the Association, as they may deem proper, from their own membership or from the membership of the Association.

Section 5. The Board of Directors shall have the authority to fill vacancies that may occur among the offices of the Association, such appointees to serve during the remainder of the unexpired term of the office in question.

#### ARTICLE III—DUTIES OF OFFICERS

Section 1. The President of the Association shall preside at all meetings of the Association and meetings of the Board of Directors and shall perform such other duties as pertain to that office. The Vice-President shall perform the duties of the President in the absence of the President.

Section 2. The Secretary-Treasurer shall have charge of the business management of the Association, shall have custody of the books and records of the Association, keep the minutes of all meetings of the Association and the Board of Directors, maintain a list of all members and subscribers, keep the funds of the Association, and make disbursements therefrom when properly authorized.

Section 3. The Journal Management Committee shall have the general supervision of the Journal. The Journal Editor under the general supervision of this Committee, shall have direct charge of all editorial details of the Journal.

Section 4. The Board of Directors shall pass upon all applications for divisions, sections, and student branches of the Association.

Section 5. The Board of Directors shall have full control of the business of the Corporation, and the title to all property and funds of the Association shall be vested in the Board of Directors. The Board of Directors shall have all the rights and powers vested in the Corporation by the laws of the District of Columbia.

#### ARTICLE IV—ELECTION OF OFFICERS

Section 1. On or before September 10, the Secretary shall mail to

each member a blank on which he shall be entitled to express his choice for each office to be filled. This blank shall give the report of the Nominating Committee which shall suggest the names of two members for each office to be filled. All ballots shall be counted by the Secretary and later verified by the President. In case no candidate has a majority by the first ballot, the tie shall be broken by the Board of Directors.

#### ARTICLE V—MEETINGS

Section 1. Meetings of the Association shall be held at the time and place fixed by the Board of Directors, but not less than one each calendar year shall be held. Notice of the time and place of meetings of the Association shall be given to all members not less than four weeks prior to the date of the meeting.

Section 2. Meetings of the Board of Directors shall be held upon call of the President provided, however, that not less than 10 days notice of such meeting shall be sent to each member of the Board of Directors.

Section 3. The quorum of any meeting of the Association shall consist of not less than ten per cent (10%) of the membership.

#### ARTICLE VI—ORGANIZATION OF DIVISIONS,

##### SECTIONS AND STUDENT BRANCHES

Section 1. Professional groups based on geographical considerations to be known as divisions of the Association and to be organized by the members of the Association may be authorized by the Board of Directors when such action shall seem expedient. The officers of the division shall be a chairman, and such other officers as are provided by the division.

The divisions shall have the right to make by-laws for their own government which shall not be inconsistent with the charter and the by-laws of the Association.

Membership in divisions of the Association is open only to those regularly elected members of the Association.

Any division may raise or collect funds to be expended for its own purpose.

Section 2. Professional groups based upon specialized interests to be known as sections of the Association and to be formed by not less than ten members may be authorized by the Board of Directors when considered for the best interests of the Association.

Such sections may elect their own officers and may make rules for their own guidance not inconsistent with the charter and by-laws of the Association.

Section 3. Student branches at any agriculture college may be authorized by the Board of Directors on petition from at least ten students regularly enrolled in a four-year course in agriculture and majoring in

some phase of the dairy industry when their petition is recommended by two department members who are members of the Association.

#### ARTICLE VII—JOURNAL OF DAIRY SCIENCE

Section 1. The Journal of Dairy Science, published by the Association, shall be sent to each member, provided his dues are paid by January 10.

#### ARTICLE VIII—AMENDMENTS

Section 1. These by-laws may be amended at any meeting of the Association by an affirmative vote of two-thirds of those members present, provided that not less than 10% of the membership is present at the meeting.

All amendments must be referred to the Board of Directors for its recommendation prior to the final action by the Association. The Board of Directors may, at its discretion, submit proposed amendments which have received the approval of the Board, to the members of the Association for vote by mail. In such case, an affirmative vote of two-thirds of all voting, and which shall not be less than a majority of the membership, shall be necessary for approval.

Mr. O. F. Hunziker, chairman of the Journal Management Committee, gave a summary of the report which he had submitted to the Board of Directors. Upon motion duly seconded this report was approved.

Vice-President Weaver, chairman of a committee appointed to formulate a procedure whereby the sections and groups of the Association may secure Association approval of their actions before such actions are released for publication, submitted the report for the committee and upon motion duly seconded it was adopted. (Report will be found in minutes of the Board.)

J. A. Nelson submitted his report on junior chapters of the A. D. S. A. Upon motion duly seconded the report was adopted.

H. A. Ruehe, chairman of the Nominating Committee, submitted the following report:

The Committee on Nominations present the following nominations:

#### *Vice-President*

E. S. Guthrie, Ithaca, N. Y.  
J. A. Nelson, Bozeman, Montana

#### *Director*

M. E. Parker, Chicago, Ill.  
W. D. Dotterer, Chicago, Ill.

#### *Director*

C. S. Rhode, Urbana, Ill. J. W. Linn, Manhattan, Kansas

Respectfully submitted,

H. A. RUEHE, *Chairman*  
D. R. THEOPHILUS

H. F. JUDKINS  
J. B. FITCH

K. M. RENNER

Upon motion duly seconded the report was adopted and the committee discharged.

In the absence of Mr. H. P. Davis, Mr. H. C. Jackson submitted the report of the Resolutions Committee. The following report was read:

#### RESOLUTIONS

Whereas the American Dairy Science Association assembled at their thirty-third annual meeting at the Ohio State University has enjoyed a splendid program and many fine courtesies extended by faculty members of the various departments and their wives:

Therefore, Be it resolved:

That the membership of the American Dairy Science Association, their wives and families, wish to express their grateful appreciation to the staff of the Ohio State University, their wives and other organizations responsible for this entertainment.

Whereas the Borden Company has seen fit to continue their awards for outstanding research in the field of dairying:

Therefore, Be it resolved:

That the American Dairy Science Association again express its appreciation of this interest in dairy research.

Whereas from time to time variations in the manipulation of the Babcock test have been proposed:

Therefore, Be it resolved:

That the American Dairy Science Association make a comprehensive examination of the Babcock procedure for determining fat in milk and cream with the purpose of refining the technique, obtaining greater accuracy, reliability and uniformity without undue increase in complexity.

Whereas the Council on Foods of the American Medical Association has added oleomargarine to the approved list of foods and has removed butter from the same, and whereas all results of scientific investigation have shown the nutritional superiority of butter:

Therefore, Be it resolved:

That the American Dairy Science Association request the American Medical Association to request its Council on Foods to reconsider this action which may have such far reaching effects on a basic food industry.

Whereas the untimely passing of three of our esteemed members, Prof. E. B. Pitts of the Pennsylvania State College, Prof. Rush B. Locke of the Colorado State College, and E. S. Raven of the Raven Creamery, Portland, Oregon, has occurred during the past year, and whereas a keen sense of loss is felt by members of the American Dairy Science Association.

Therefore, Be it resolved:

That a recognition of this feeling be spread upon the records of the Association, and that the Secretary forward a copy of this resolution to the families of the deceased.

Whereas Dr. A. C. Dahlberg has asked to be relieved as editor of the JOURNAL OF DAIRY SCIENCE:

Therefore, Be it resolved:

That the membership of the American Dairy Science Association expresses its deepest regret that Doctor Dahlberg has felt it necessary to resign as editor of the JOURNAL OF DAIRY SCIENCE, a position which he has so bril-

liantly filled. The American Dairy Science Association further expresses its keenest appreciation of Doctor Dahlberg's ability as a scholar and scientist and its tribute to the great service rendered by him to this Association as Journal Editor.

Whereas the New York State Agriculture Experiment Station through its director has permitted Dr. A. C. Dahlberg to act through these years as Editor of the JOURNAL OF DAIRY SCIENCE:

Therefore, Be it resolved:

That the American Dairy Science Association express to Director Parrott and through him to the station, in regretfully accepting Doctor Dahlberg's resignation, its very great appreciation of the facilities provided and for giving the cooperation which has been so generously and graciously accorded the Journal Editor's office.

Respectfully submitted,  
H. C. JACKSON  
FORDYCE ELY  
E. V. ELLINGTON  
H. P. DAVIS

Upon motion duly seconded the report was adopted and the committee discharged.

The Secretary read the minutes of the Board of Directors and upon motion duly seconded the minutes were approved, and all action of the Directors during the past year were authorized and approved.

#### MEETING OF THE BOARD OF DIRECTORS

#### AMERICAN DAIRY SCIENCE ASSOCIATION

COLUMBUS, OHIO, JUNE 13, 1938

A meeting of the Board of Directors of the American Dairy Science Association was held in Townshend Hall, Monday evening, June 13, at 7:30 P.M. Present:—Pres. H. W. Gregory, Directors E. G. Hood, C. R. Gearhart, J. A. Nelson, C. E. Wylie, E. V. Ellington, H. Macy, and R. B. Stoltz. Absent:—Vice-President Earl Weaver and Director R. R. Graves.

O. F. Hunziker, chairman of the Journal Management Committee, appeared before the Board and gave a very comprehensive report discussing Journal finances (the additional annual cost for publication of abstracts of the literature on dairy cattle); recommendations for successor to Journal editor; budget for the office of editor; publication of abstracts of literature on dairy cattle; abstracts of annual meeting (one month in advance of the date of the meeting)—“Incidentally an unfortunate error occurred in the paging of the May issue of the Journal containing the abstracts of the annual meeting. The entire issue is paged for abstracts of literature instead of for main Journal text, as it should have been. This places the program and the original papers (abstracts of annual meeting) in the Abstract Section, which regularly consists of previously published material.

“This error which occurred during the Editor's absence due to serious illness, obviously cannot be undone. It is proposed, however, that this

material in the May Journal be considered for this year only in the Abstract Section. At the end of the year it will then be indexed in both the Abstract Section and in the section for original articles. While this makes the index for the section of the original articles somewhat cumbersome, confusion can be and will be avoided by using the letter "A" in front of the page and placing a footnote at the bottom of each page, stating that "A" refers to the Abstract Section.

"We propose also to make an announcement of this error at the General Session of this annual meeting, explaining to the membership the manner in which the matter will be taken care of. It was suggested that an insert be placed in the July issue making a statement regarding the renumbering of the pages."

Mr. Hunziker further discussed bids for printing the Journal; Reprints of chemical and bacteriological methods—"In the past the Chairman of the Committee on Laboratory Methods ordered a supply of reprints for distribution among members who wanted copies at 50¢ per copy, and the cost of these reprints was charged to the Journal with the understanding that after the committee chairman had sold a sufficient number, the Journal would be reimbursed out of such sale. As time went on the chairman developed a small fund which was maintained by the Committee to purchase reprints as issued.

"This appeared to work fairly satisfactorily but this service could obviously be improved by assisting the members in ordering reprints direct from the source of supply. In order to place this whole matter of reprints of chemical and bacteriological methods on a somewhat more business-like basis, your Committee recommends:

"1. That those interested in reprints on laboratory methods order the same direct from the Secretary-Treasurer, accompanying their order with remittance of the price per copy.

"2. That this change take effect at once.

"3. That announcement of this change be made at the general session of this annual meeting as well as in each issue of the Journal in which laboratory methods appear.

"4. That the chairmen of the Committees on Laboratory Methods be requested to transfer the fund that they may have accumulated from former sales of reprints to the Secretary-Treasurer.

"5. That the reprints in the hands of the chairman be transferred to the office of the Secretary and their availability be called to the attention of the members."

Further discussion covered insurance on back numbers of the Journal; publication of committee reports as follows:—"The Suggestion that committee reports of importance be published in the Journal originated with our Editor, Dr. Dahlberg. Your committee is heartily in favor of this

suggestion and we recommend it to this Board for consideration and approval. In the case of the Committee on Laboratory Methods, for instance, it is mandatory by vote of our Association, that reports after they have been accepted, be published in the Journal.

"There are other committee reports which are more or less official for our Association, and which are sufficiently important to merit a place in the Journal for similar reasons as prominence is given to any other original article. This would be true of reports for instance on dairy products standards, breed relation score cards and many other reports of similar importance.

"Your Committee, therefore, recommends that the Board of Directors, in case they are favorable to this suggestion, direct the committees of the Association to send their reports, after they have been finally approved by the Association, to the Journal Editor for publication in the Journal."

The concluding point concerned donations to other Journals.

Respectfully submitted,

A. A. BORLAND

R. B. STOLTZ

O. F. HUNZIKER, *Chairman*

Mr. Ellington moved and Mr. Gearhart seconded that the Board of Directors accept the resignation of Dr. Dahlberg and send him our regrets that he could not be present and to send the Board's appreciation of his services. Motion carried, and the following telegram was sent to him:

"It is with sincere regret that we received and accepted your resignation as Editor of the Journal of Dairy Science. We deeply deplore your unavoidable absence from our annual meeting due to ill health that is preventing you from receiving, in person, our expression of appreciation and our tribute to the great service you have rendered our Association as Journal Editor. Accept our earnest good wishes for your speedy and complete recovery."

BOARD OF DIRECTORS

*American Dairy Science Association*

It was moved by Mr. Wylie and seconded by Mr. Ellington that Dr. T. S. Sutton be employed as Editor of the Journal beginning July 1, 1938. Motion carried unanimously.

Mr. Nelson moved and Mr. Hood seconded that the Secretary be instructed to write to the Director of the New York State Agricultural Experiment Station the appreciation of this Board for making it possible for Doctor Dahlberg to serve as Editor and permitting clerical assistance for carrying on the work of the Editor of the Journal of Dairy Science during the past ten years. Motion carried. The following letter was sent:

"In accepting with regret Dr. A. C. Dahlberg's resignation as Editor of the Journal of Dairy Science, we desire to express to you our sincere appreciation and gratitude for the high privileges, helpful facilities and generous cooperation that the New York State Agricultural Experiment Station so freely accorded our Journal Editor's office for the lasting benefit of the Dairy industry."

BOARD OF DIRECTORS  
*American Dairy Science Association*

Mr. Macy moved and Mr. Ellington seconded that the report pertaining to "reprints of chemical and bacteriological methods" be approved. Motion carried. Mr. Macy moved and Mr. Wylie seconded that the recommendation of the Journal Management Committee on publication of committee reports be approved as amended. Motion carried. Secretary Stoltz moved and Mr. Macy seconded that the report of the Journal Management Committee referring to donations to other Journals be approved. Motion carried. Mr. Macy moved and Mr. Wylie seconded that the report of the Journal Management Committee as amended be accepted. Motion carried.

The President appointed a committee to draw up a set of by-laws to replace our present by-laws, consisting of S. M. Salisbury, R. R. Graves, C. L. Roadhouse and R. B. Stoltz. These by-laws were submitted to the Board of Directors, and upon motion by Mr. Nelson and seconded by Mr. Wylie, it was recommended that the by-laws, as amended, be submitted to the membership for their approval. Motion carried.

The by-laws will be found printed in the minutes of Business Meeting.

President Gregory had previously appointed Mr. Macy to make a study of the rules concerning the Borden Award. He gave a report to the Board of Directors telling of how other associations handled similar awards. Mr. Wylie moved and Mr. Nelson seconded that President Gregory appoint a committee of three to draw up rules for the Borden Award. Motion carried. President Gregory appointed Mr. Macy, Mr. H. A. Ruehe and F. B. Morrison. The Board then adjourned.

MEETING OF THE BOARD OF DIRECTORS  
COLUMBUS, OHIO, JUNE 14, 1938

A meeting of the Board of Directors of the American Dairy Science Association was held in Campbell Hall, Room 203, Tuesday, June 14, 1938, at 4:30 P.M. Present:—Pres. H. W. Gregory, Vice-Pres. Earl Weaver, Secretary Stoltz, E. G. Hood, C. E. Wylie, C. R. Gearhart, R. R. Graves, E. V. Ellington, and J. A. Nelson. Absent:—H. Macy.

Professor C. N. Shepardson of Texas appeared before the Board and extended the Association an invitation to hold their 1940 annual meeting in Texas. No action taken.

P. F. Sharp, chairman of the Committee on the Standardization of Market Milk, appeared before the Board and recommended the approval of the following report:

REPORT OF COMMITTEE ON STANDARDIZATION OF MARKET MILK  
OHIO MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION,  
JUNE 14-17, 1938

A committee of the American Dairy Science Association was appointed by H. A. Ruehe and continued by R. R. Graves, Presidents of the Association, to make recommendations regarding the mechanical standardization of market milk. At the meeting held in Lincoln, Nebraska, June, 1937, the recommendation of the committee was adopted, that a copy of its report be given to the members of the American Dairy Science Association as a preliminary to its consideration at the Ohio meeting.

*Believing:* that laws and regulations prohibiting the standardization of the fat content of market milk are unsound in principle as a means of protecting the consumer, and that legalization of the mechanical standardization of the fat content of market milk would remove an important economic restriction which operates to the mutual disadvantage of the farmer the distributor and the consumer; the American Dairy Science Association approves and recommends in principle the following:

*First:* the legalization of the alteration of the fat content of market milk by mechanical standardization either up or down, by an amount not to exceed 0.6 per cent of fat, provided that all products used in standardization be at least equal in sanitary and physical quality and be held not longer than the milk to be standardized, and provided either that the minimum guaranteed fat content be stated on the label, or that classes or grade designations based on fat content be established and the class or grade designations corresponding to the legally established minimum levels of fat content be stated on the label.

*Second:* that the legal minimum for the solids-not-fat of milk be 8.15 per cent.

*Third:* That the legalization of the mechanical standardization of the solids-not-fat content of market milk by the addition of dried or condensed milk is inadvisable at this time.

A. D. BURKE  
W. D. DOTTERER  
J. H. FRANDSEN  
C. L. ROADHOUSE  
P. F. SHARP, *Chairman*

Upon motion duly seconded the report was approved and referred to the membership for their action. The motion carried not unanimously.

The meeting then adjourned.

MEETING OF THE BOARD OF DIRECTORS  
COLUMBUS, OHIO, JUNE 15, 1938

A meeting of the Board of Directors of the American Dairy Science Association was held in Townshend Hall, Wednesday, June 15, 1938, at 4 P.M. Present:—President H. W. Gregory, Vice-Pres. Earl Weaver, Secretary R. B. Stoltz, Directors J. A. Nelson, E. G. Hood, R. R. Graves, C. R. Gearhart and E. V. Ellington. Absent:—Directors H. Macy and C. E. Wylie.

The Secretary-Treasurer submitted the financial report which was sent to all directors in the month of February. Upon motion by Mr. Weaver and seconded by Mr. Graves this report was accepted and referred to the Auditing Committee. The report of the Auditing Committee was then read. Upon motion duly seconded the report of the Auditing Committee was accepted and referred to the Association. (Auditing Committee report may be found in minutes of the business meeting.)

G. M. Trout then appeared before the Board and requested the Board to give authority to the Dairy and Ice Cream Machinery and Supplies Association to publish the report of the judging of dairy products prepared by Mr. Trout in the Association Quarterly magazine. Upon motion duly seconded the Board of Directors approved the request.

Mr. Gearhart moved and Mr. Nelson seconded that the time of meeting at Washington and Idaho in 1939 be the week of June 26. Motion carried.

Mr. Nelson then reported on Student Branches of the A.D.S.A. Mr. Nelson moved and Mr. Graves seconded that the President appoint a committee of three to formulate a plan of junior chapters of the A.D.S.A. Motion carried. The President appointed Mr. Nelson of Montana, Mr. Ellington of Washington and Mr. Borland of Pennsylvania.

Mr. Weaver was then called upon to present a report of the committee appointed to formulate a procedure for various sections and groups of the Association. The report follows:

TENTATIVE REPORT

A committee consisting of J. A. Nelson, C. R. Gearhart and Earl Weaver was appointed by Pres. R. R. Graves in June 1937 to formulate procedure for various sections and groups of the Association to use in securing Association approval of such actions of these sections and groups as require such approval prior to their release for publication.

The committee proposes:

(1) Policy

- (a) The action of any section or group which establishes grades or standards, prescribes methods for the conduct of tests or determinations, establishes regulation or in any other manner possesses

general interest may be published in such manner as deemed advisable subject to the approval of the Association in accordance with the provisions below.

- (b) Association approval shall be construed as the favorable action of the membership in annual meeting or in cases of emergency of the favorable action of the Board of Directors. The decision as to the existence of an emergency shall rest with the President of the Association.
- (c) No action of any section or group shall be purported to carry the Association approval until such approval has been granted.
- (d) No action of any section or group shall be published in the Journal of Dairy Science until the Association shall have given its approval for such publication.
- (e) Final judgment in respect to the advisability of publication in the Journal of Dairy Science of any action of the Association or of its sections and groups shall rest with the Board of Directors.

(2) Organization

- (a) At the beginning of the Association year on October 1 the President shall appoint a general resolutions committee of five members to serve for that year.
- (b) At the opening general session of each annual meeting the President shall have announced the time and place of the first meeting of the general resolutions committee.
- (c) The chairman of each section and group shall be notified in advance of such subsequent meetings of the general resolutions committee as its chairman shall call during the period of the annual meeting.
- (d) The general resolutions committee may submit a partial report at any general session during the annual meeting.

(3) Procedure

- (a) It shall be the duty of the secretary of each section or group to transmit to the general resolutions committee or some member thereof, the action of his section or group upon which it is desired to receive Association approval.
- (b) The general resolutions committee shall exercise its authority to reject the action thus transmitted or to submit it for the approval of the Association.
- (c) Any member may submit from the floor of any meeting any matter upon which he desires to obtain Association action.

Signed: EARL WEAVER, *Chairman*  
C. R. GEARHART  
J. A. NELSON

Upon motion by Mr. Nelson and seconded by Mr. Ellington the report of the committee was approved.

MEETING OF THE BOARD OF DIRECTORS  
COLUMBUS, OHIO, JUNE 16, 1938

A meeting of the Board of Directors of the American Dairy Science Association was held in Townshend Hall, Thursday, June 16, 1938 at 1:00 P.M. Present:—Pres. H. W. Gregory, Vice-Pres. Earl Weaver, Secretary Stoltz, E. G. Hood, J. A. Nelson, R. R. Graves, C. R. Gearhart. Absent:—C. E. Wylie and H. Macy.

The Program Committee gave the following report:

The Program Committee consisted of E. N. Shultz, W. E. Krauss, C. J. Babcock, with T. S. Sutton as Chairman; S. I. Bechdel and H. P. Davis were advisory members. The change in personnel of this committee from that of previous committees was made following a recommendation of the Program Committee of 1937. The present arrangement did not remove all the objections to the previous method of selecting the personnel of this committee. The other members of the committee are still located at considerable distance from the place of meeting. This year we were fortunate in having the chairman of the Production Section in this State and the chairman of the Manufacturing Section designated a member of the local Department of Dairy Technology to act for him in selecting and organizing papers for the Manufacturing Section.

The present Program Committee was handicapped for time. The Board of Directors adopted a resolution presented by the 1937 program committee, setting April 15 as the latest day for receiving titles and abstracts. A report of this action was published in the December issue of the Journal. The Journal Editor called for the program material on April 15, in order that it could appear as the May issue of the Journal.

Prior to April 15, the general program was organized and a definite time allotment set for papers voluntarily submitted. Papers were slow coming in; however, by the evening of the 15th, more papers than could be included in the time allotted were at hand. The recommendations of the 1937 program committee adopted by the Board of Directors provided means for making our decision on papers which were not included in the program. Papers which were received late (after April 15) were either included among those read by title or returned to the authors. Our program was organized, copied and in the mail to the Editor, April 16.

The present committee set the precedent in not including on the program all the papers submitted. The majority of the papers in the "read by title" group were so placed because of late arrival or duplication of authorship. For real effectiveness of the program, most eliminations should be made on the basis of the quality of the offering. We trust that future committees will be able to make more of their decisions on this basis.

The present program includes as special features, a symposium on nutrition, an education section and a general session at the Ohio Agricultural Experiment Station. Because of a lack of room facilities at the Experiment Station, it was impossible to schedule sectional meetings, and a program of general interest with invited speakers was decided upon. Our Wooster guests will have some time to inspect the experimental work in progress at the Station.

Because of the interest in artificial insemination an additional session of the Extension Section was scheduled for the discussion of this subject.

In order that the program committee may have more time for the careful consideration of the abstracts in making up the program, we recommend, that the last date for receiving abstracts be set some time in advance of the time the program must be submitted to the Editor. Such a date should be worked out by the chairman of the Program Committee and the Editor.

E. N. SHULTZ

C. J. BABCOCK

W. E. KRAUSS

T. S. SUTTON, *Chairman*

The meeting of the Board of Directors was then adjourned.

AMERICAN DAIRY SCIENCE ASSOCIATION PRESENTED  
BORDEN AWARDS TO K. G. WECKEL AND  
W. E. KRAUSS

AT THE  
ANNUAL BANQUET

NEIL HOUSE  
COLUMBUS, OHIO, JUNE 16, 1938

S. M. Salisbury, toastmaster, presented the officers and distinguished guests. He then introduced H. A. Reuhe, chairman of the Dairy Manufacturing Awards Committee, who made the following statement:

The Committee on the Borden Award in Dairy Manufacturers submits the following report:

According to the rules adopted by the Executive Board of the American Dairy Science Association, the Borden Award in Dairy Manufactures is awarded for outstanding research in the processing field such as improvement in equipment or methods in the handling of milk or cream and the production of milk products. The recipient must not have reached his fortieth birthday.

Your committee adhered rigidly to these rules in its consideration of nominees.

Your committee is pleased to announce that it has selected Dr. Kenneth G. Weckel, Dept. of Dairy Industry, University of Wisconsin, as the recipient of the Borden Award in Dairy Manufactures for the year 1938.

Doctor Weckel was selected on the basis of the significant contributions which he has made in the application of irradiation to the commercial dairy industry. He is responsible for the development of apparatus which has made the irradiation of milk commercially possible.

Doctor Weckel was born in Canton, Ohio, in 1906. He received his undergraduate training at the University of Wisconsin and was graduated with the Bachelor of Science degree with honors in 1931. He was granted the Master of Science degree in 1932, and the degree of Doctor of Philosophy in 1935 by this same institution.

Doctor Weckel has devoted his attention to the study of light and its relation to the vitamin D potency of dairy products. He is an authority in this field. The results of his research have appeared in many articles published both in scientific journals and trade papers. In all of his writings, as in his research, he has kept the practical application of his studies in mind, making it possible for the commercial industry to keep abreast with his scientific findings.

Dr. Kenneth G. Weckel has brought honor to himself and to the institution of which he is a member through his efforts and his desire to serve the dairy industry to the best of his ability.

Respectfully submitted,

M. E. PARKER

H. L. RUSSELL

H. A. RUEHE, *Chairman*



KENNETH G. WECKEL

"Mr. Wentworth, it is now my pleasure to present to you as the recipient of this Award, Dr. Kenneth G. Weckel."

Mr. W. A. Wentworth, representing the Borden Company, then spoke as follows:

"Dr. Weckel, on behalf of The Borden Company I present this medal to you which is given in recognition of the fine work you have done in research.

"So that you and all those present may know the message which is conveyed with this medal, I should like to read from its reverse side:

Award for Outstanding Achievement in Dairy Manufacturing Research to *Kenneth G. Weckel, 1938*, by  
Direction of American Dairy Science Association.

"Along with the medal goes a financial reward which we trust will be of value to you and possibly afford you an opportunity to possess some things which might not otherwise be available to you.

"The purpose of this award in addition to rewarding and recognizing you as an individual is to stimulate in others, the hundreds of them who are engaged in similar work throughout the United States and Canada, further constructive research in the dairy manufacturing field for the advancement of the industry.

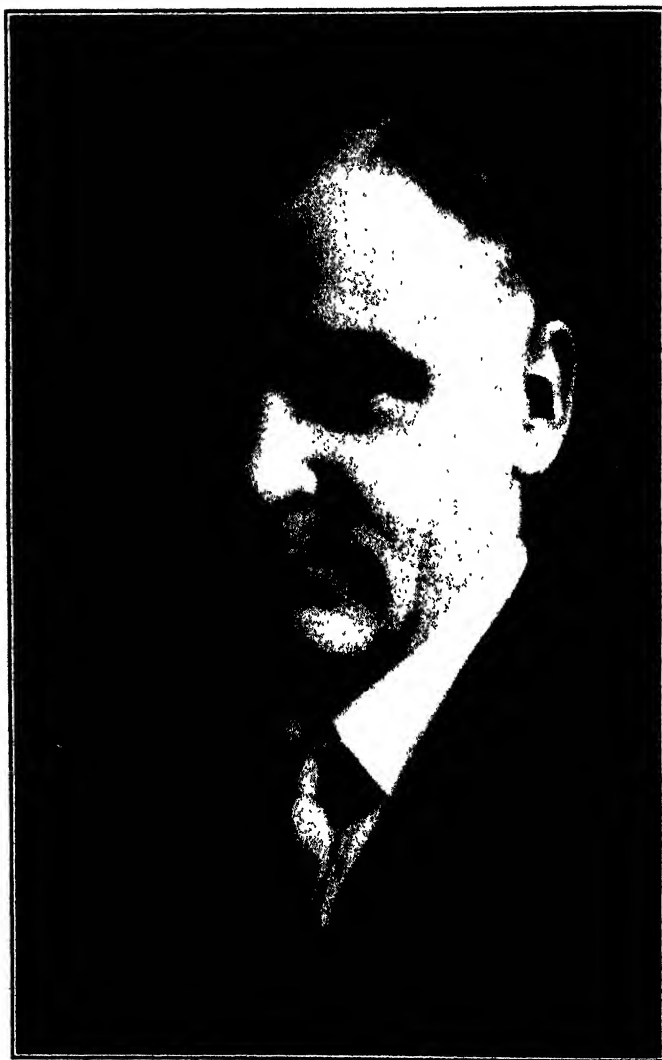
"Dr. Weckel again on behalf of The Borden Company, I wish to congratulate you upon your own attainments which tonight are recognized by the American Dairy Science Association and particularly by those of that Association who are here tonight and who have so grandly indicated their concurrence in the grant of the award to you."

The toastmaster then introduced F. B. Morrison, chairman of the Production Award Committee, who gave the following report:

The Dairy Production Award Committee of the American Dairy Science Association, whose function it is to select the person to receive the Borden Award in the field of dairy production, has unanimously chosen Dr. William E. Krauss, of the Ohio Agricultural Experiment Station at Wooster, Ohio, for this honor on account of his important and able investigations in this field.

It was no small task for the members of the Award Committee to select the person to receive this award from the several who had been recommended for our consideration by the Nominating Committee or who had been proposed to us directly by other members of our Association. This was because several men had been suggested who had done important research work of a high grade in the field of dairy production.

Each member of the Award Committee considered, entirely independently, the information available concerning each person who had been nominated, including his research publications. Without any knowledge



WILLIAM E. KRAUSS

of how the other members of the Committee had ranked the nominees, each of us arrived at a definite ranking. Fortunately, when we compared by correspondence our decisions, we found that we had each placed Doctor Krauss in first rank.

We selected Doctor Krauss for this honor primarily because of the extensive investigations in which he has been the leader, upon the nutritive value of milk and upon factors affecting its quality and value. Doctor Krauss has not only obtained in these researches much information which is of great importance to the dairy industry, but he has also carried this information to the dairy industry by means of publications of a popular nature and addresses before various dairy organizations.

Among the important investigations by Doctor Krauss in this field are the following: The comparisons of various methods of increasing the vitamin D potency of milk, including studies of the cost with various methods. Extensive studies on the effects of pasteurization on the nutritive properties of milk in which it was concluded that "The nutritive deficiencies of pasteurized milk (vitamin B and C) can be readily overcome by proper dietary control, and the continued use of pasteurized milk offers no serious problem from the food standpoint." Investigations of the effect produced upon the carotene content and other biological properties of milk, by feeding legume and grass silage.

Due consideration was also given to the joint investigations conducted in the field of dairy production by Doctor Krauss and his colleagues—Professors Hayden, Sutton, Bethke, Monroe and Perkins. These have included the extensive studies on the vitamin A and carotene content of the butterfat produced by various breeds of cows, on the use in dairy rations of fish meal and products containing fish meal, on methods of raising dairy calves on dry calf meals, and on such practical management problems as box stalls vs. tie and stanchion stalls for milk cows.

On account of the services of Doctor Krauss to the dairy industry in these several investigations, the Award Committee takes pleasure in presenting him to Mr. Wentworth, of The Borden Company, to receive the Borden Award in Dairy Production for 1938.

H. B. ELLENBERGER

C. W. LARSON

F. B. MORRISON, *Chairman*

"Mr. Wentworth, it is with pleasure that I present to you Dr. W. E. Krauss, the recipient of the Dairy Production Award."

Mr. Wentworth then spoke, "On behalf of The Borden Company and with a feeling of personal pride because of my acquaintance with you, I wish to present you with this much deserved award for the work you have done in scientific research.

"Like I did in the preceding presentation, I should like to read to you and for the benefit of those gathered, the inscription on the reverse side of the medal:

Award for Outstanding Achievement in Dairy Production Research to *Dr. William E. Krauss, 1938*, by Direction of American Dairy Science Association.

"What I said in the previous presentation regarding the recognition of its recipient and the hope which The Borden Company has that it will stimulate further research and progress in this industry, applies equally in your case.

"The Borden Company congratulates you on the success you have attained and I add my own best wishes."

The presentation of Awards was completed at 9:00 P. M., and the rest of the evening was given over to visiting, card playing and dancing.

#### GENERAL PROGRAM

OHIO AGRICULTURAL EXPERIMENT STATION  
WOOSTER, OHIO, JUNE 17, 1938

Mr. C. C. Hayden introduced Vice-Pres. Weaver at 10:45 A. M., as chairman of the morning's program. Director Edmund Seerest of the Ohio Agricultural Experiment Station was then introduced and gave an address of welcome. The program was followed as scheduled by the program committee except that in the absence of Dean Anthony, Mr. Balser read his paper.

Chief O. E. Reed of the Bureau of Dairy Industry was called upon and gave a very short and interesting message regarding the benefits one may derive by attending these meetings.

There were 154 in attendance.

After lunch was served a visit was made to observe the herd, farm and laboratories.

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## A STUDY OF THE RELATION OF THE FEED CONSUMED BY THE COW TO THE COMPOSITION OF MILK FAT AND THE PROPERTIES OF BUTTER\*†

O. J. HILL‡ AND L. S. PALMER

*Divisions of Agricultural Biochemistry and Dairy Husbandry,  
University of Minnesota, University Farm, St. Paul*

The fact that cows can produce butterfat from rations low in ether extract has been shown by Jordan and Jenter (1), Hills (2), Lindsey (3) and Buschmann (4). Hansson and Olofsson (5) and Magi (6) have reported hard butter following winter feeding with hay and grain. Hansen and Stensberg (7) classified oats with the feeds producing normal butter and barley in the group producing hard butter. Many workers present data concerning the influence of feed consumed by the cow on the composition of butterfat. Few studies have been conducted to determine the relationships between observed changes in butterfat composition and its hardness due to feeding practice.

### EXPERIMENTAL METHODS

Seven-day feeding periods were used except for part of the oil feeding trials in which six days were used. Although samples were taken at the end of each period, the roughage feeding was extended through two or more periods in order to allow time for the animals to reach equilibrium. Cows producing more than one pound of butterfat daily were used as experimental subjects. A basal mixture consisting of 70 parts barley and 30 parts bran, by weight, was used as concentrate unless substitutions or additions were made, as during the oats, corn, or oil feeding trials. Samples of butter and butter oil were prepared at the end of each feeding period as described by Coulter and Hill (8). Saponification value, iodine absorption value, Reichert-Meissl numbers, and melting points were made for butterfat samples. Hardness of butter and butterfat was determined according to the method described by Coulter and Hill (8). Butter hardness was not

\* Received for publication March 16, 1938.

† This paper represents a portion of the thesis presented by O. J. Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota. Published with the approval of the Director as Paper No. 1600 Journal series, Minnesota Agricultural Experiment Station.

‡ Now at Washington State College, Pullman, Washington.

determined for all samples since the correlation coefficient between hardness of butter and butterfat for 28 samples was + 0.905.

*The effect of changing from the herd ration to the experimental rations consisting of barley, bran and hay (timothy or alfalfa)*

Two groups of two cows each, consisting of one Jersey and one Holstein, were used in this experiment. The herd ration included a balanced grain ration, alfalfa hay and corn silage. Commercial casein was added to the timothy hay ration in sufficient quantities to properly balance the ration. A like amount of casein was fed to the group receiving alfalfa to equalize any effect the casein might exert. The experimental rations provided .23 pounds less fat daily per cow than the herd ration. As shown in Table 1,

TABLE 1

*The effect of changing from the herd ration to the experimental rations on the fat constants and the hardness of butterfat*

Period	Hay	Chemical constants			Physical constants	
		Saponification value	Iodine value per cent	Reichert-Meissl number	Melting point °C.	Hardness grams
Group I	1 Herd <sup>1</sup>	236.1	29.34	30.32	32.55	1342
	2 Timothy	231.8	31.71	27.11	32.90	1307
	3 Timothy	231.4	31.43	26.52	33.15	1439
	4 Timothy	234.6	28.15	29.48	32.90	1532
	5 Timothy	235.2	25.48	28.84	33.90	1992
	6 Timothy	236.4	25.01	30.04	34.00	2044
	7 Timothy	235.9	25.05	29.37	34.25	2172
Difference						
Period 1 to 7 (19 days)		- 0.2	- 4.29	- .95	+ 1.70	+ 830
Group II	1 Herd <sup>1</sup>	233.5	32.40	27.94	32.05	1205
	2 Alfalfa	233.8	29.40	28.43	33.45	1869
	3 Alfalfa	234.0	28.79	28.64	33.10 *	1424
	4 Alfalfa	232.2	30.00	26.47	34.20	1610
	5 Alfalfa	233.1	26.20	25.57	34.85	2321
	6 Alfalfa	232.7	27.32	26.90	34.82	2216
	7 Alfalfa	234.1	27.70	26.87	34.00	1872
Difference						
Period 1 to 7		+ 0.6	- 4.70	- 1.07	+ 1.95	+ 667

<sup>1</sup> Fed on the regular herd grain ration, alfalfa hay, corn silage.

there was considerable change in chemical and physical constants in the butterfat from both groups of cows. In Group I there was a drop in iodine value of 4.29, in Reichert-Meissl number of 0.95, while melting point and hardness increased 1.70 degrees C. and 830 grams respectively. In Group II there was a decrease of 4.70 in iodine value, of 1.07 in Reichert-

Meissl number, and an increase of 1.95 degrees C. in melting point and 667 grams in hardness. Changes in saponification value were practically negligible. All the butters for period 5, 6, and 7 were scored "hard and brittle."

*Influence of alfalfa hay, timothy hay, or combinations of these hays with beet pulp*

Results for groups I and II, shown in Table 2, are a continuation of Table 1, except that they are averages for three periods. The groups were reversed in order to determine the response of each group to each roughage. Variances in the data are not sufficient to ascribe a specific effect to any one of the combinations fed. All butters during these periods were pronounced hard and dry by the judges.

*Influence of heavy alfalfa feeding*

In a further attempt to establish the effect of alfalfa on the chemical constants and hardness of butterfat, very high levels of alfalfa were fed to cow 419, a Holstein. This cow consumed 85 per cent of her total digestible nutrients in the form of alfalfa hay. Table 2 demonstrates a considerable

TABLE 2

*The effect of feeding timothy hay, alfalfa hay, and beet pulp on the chemical and physical constants of butterfat (averages)*

	Roughage	Chemical constants			Physical constants	
		Saponi- fication value	Iodine value per cent	Reichert- Meissl number	Melting point °C.	Hardness grams
Group I	Timothy hay	236.2	25.03	29.71	34.12	2108
	Alfalfa hay	234.2	26.30	28.41	34.30	2178
	Alfalfa hay and beet pulp	233.5	27.21	27.61	33.64	1943
Group II	Alfalfa hay	233.4	27.51	26.88	34.41	2044
	Timothy hay	233.6	27.93	26.71	34.02	1813
	Timothy hay and beet pulp	234.1	28.35	25.96	34.12	1829
Cow 419	Normal alfalfa	232.9	31.49	29.65	...	1253
	Heavy alfalfa <sup>1</sup>	230.8	35.85	28.01	...	1001
	Normal <sup>2</sup>	229.6	34.10	28.55	...	1231

<sup>1</sup> Average three periods.

<sup>2</sup> First period following heavy alfalfa.

change in butterfat constants during the heavy alfalfa feeding periods. The iodine value increased 4.36, the Reichert-Meissl number declined 1.64, with a decrease of hardness of the butterfat of 252 grams. The body of the butter was normal in all cases, although it was somewhat harder for the

basal periods. It is evident from Table 2 that the alfalfa hay in this experiment was responsible for increasing the iodine value and decreasing the hardness of butterfat more than the alfalfa-barley-bran ration. When the alfalfa was reduced to normal amounts in the after period the hardness of the fat increased without a corresponding decrease in the iodine value. Richardson and Abbott (9) show similar increases in iodine value during alfalfa feeding, but in spite of these changes, report a "sticky" crumbly butter unless special churning procedure is followed.

*The effect of including oats and corn in the ration on the chemical constants and physical properties of butter and butterfat*

The preceding data, representing the roughage feeding experiments, showed that a hard butter of low iodine value was produced except when alfalfa was fed. The following experiments were planned in an attempt to ascertain the influence of substituting cereals higher in fat for the barley part of the ration. In all cases basal ration refers to the mixture, 70 parts barley and 30 parts bran, fed with alfalfa hay. Oats or corn replaced the barley in the basal ration during these experiments. In each case the cereal supplied 40-50 per cent of the total digestible nutrients. The experiment for cow 419 had to be altered somewhat because the oats were of such low grade, containing but 2.51 per cent ether extract compared to 3.73 per cent for the lot fed to the groups. Corn was fed with the oats to avoid

TABLE 3

*The effect of substituting oats or corn for barley on the chemical constants and hardness of butterfat (average)*

	Ration	Chemical constants			Physical constants	
		Saponi- fication value	Iodine value per cent	Reichert- Meissl number	Melting point °C.	Hardness grams
Group I	Basal <sup>1</sup>	231.7	28.31	27.47	34.63	1884
	Oats	228.7	35.51	28.81	33.12	1140
	Difference	- 3.0	+ 7.20	+ 1.34	- 1.51	- 744
	Corn	229.4	35.15	27.74	32.70	1120
	Difference	- 2.3	+ 6.84	- 0.27	- 1.93	- 764
Group II	Basal <sup>1</sup>	231.9	30.06	25.80	33.75	1757
	Oats	228.4	34.56	25.47	32.97	1372
	Difference	- 3.5	+ 4.50	- 0.33	- 0.78	- 385
	Corn	227.4	35.95	23.79	32.15	1093
	Difference	- 4.5	+ 5.89	- 2.01	- 1.60	- 664
Cow 419	Basal <sup>1</sup>	233.1	34.35	29.84	.....	996
	Oats and corn	230.5	36.37	29.30	.....	926
	Difference	- 2.6	+ 2.02	- 0.54	.....	- 70

<sup>1</sup> Barley, bran and alfalfa hay.

underfeeding as well as to increase the fat content of the ration. The data presented in Table 3 show that during the oat feeding period in Group I the saponification value, melting point, and hardness decreased 3.0, 1.51° C. and 744 grams respectively. The iodine value increased 7.20 and the Reichert-Meissl number 1.34. Corn brought about very similar changes except for the Reichert-Meissl which decreased slightly. Group II gave results in the same direction, but in less degree except for the Reichert-Meissl which decreased, especially during the corn feeding. Results for cow 419 show an increase of only 2.02 for the iodine value with no appreciable change in hardness. The butters produced during these periods were graded normal in body, as contrasted to the harder butters produced during the basal periods.

*The effect of feeding fats and oils on the chemical constants and physical properties of butter and butterfat*

The literature reveals a close relationship between the quantity of fat in the ration and the influence on the properties of butterfat. It was evident in the experiments reported in Table 4 that the oil in the corn and oats was responsible for the effects observed. Further study of feeding fats and oils possessing widely different characteristics was undertaken. The oils and fats studied were corn oil, linseed oil, cottonseed oil, tallow, butterfat and coconut oil. The iodine values of the several fats were as

TABLE 4

*The effect of feeding corn oil on the chemical and physical constants of butterfat*

Group	Ration	Amount oil fed lbs.	Chemical constants			Physical constants	
			Saponification value	Iodine value per cent	Reichert-Meissl number	Melting point °C.	Hardness grams
II	Barley, alfalfa, bran	0.0	228.7	33.19	24.40		1382
		0.3	226.4	37.43	25.12	.....	1118
		Difference	- 2.3	+ 4.24	+ 0.72	.....	- 264
III	Herd ration with corn silage and alfalfa	0.0	236.0	30.50	31.15	32.10	1347
		1.0	228.3	41.00	30.36	32.25	976
		Difference	- 7.7	+ 10.50	- 0.79	+ 0.15	- 371
IV	Same as Group III	0.0	234.2	30.28	30.60	32.55	1551
		1.0	229.0	40.20	31.93	32.60	1127
		Difference	- 5.2	+ 9.92	+ 1.33	+ 0.05	- 424
Cow 151	Barley, bran, alfalfa	0.0	232.1	32.50	29.00	.....	1396
		0.8	231.3	35.78	29.00	.....	1115
		Difference	- 0.8	+ 3.28	0.00	.....	- 281

follows: for coconut oil, 7.94; tallow, 37.48; cottonseed oil, 108.55; corn oil, 123.14; and linseed oil, 169.04. The feeding conditions were standardized so that the effect of feeding the oil could be compared with a preliminary and an after period in which no extra oil or fat was fed.

During these oil feeding periods, Groups I and II, and cows 151, a Jersey, and 419 were fed the basal grain ration and alfalfa hay. Groups III and IV, composed of three cows each, a Guernsey, a Holstein, and a Jersey, received regular Experiment Station herd ration, with corn silage and alfalfa hay, according to milk production and body weight. Groups V and VI, composed of two cows each, a Guernsey and a Jersey, were fed the Experiment Station ration and alfalfa hay according to weight and milk production. These diverse feeding practices offered an opportunity to study the effect of fat feeding under varied conditions. The fat or oil was added to the regular ration, thereby increasing the total digestible nutrients.

a. *Influence of feeding a low level of corn oil*—This experiment was designated to determine if corn oil when fed in addition to the basal ration would produce results similar to those secured when corn was fed. The digestible fat in the corn ration exceeded that in the basal ration by 0.28 pounds daily for the Jersey cows, and 0.31 pounds for the Holsteins. These amounts of oil were added to the basal ration of Group II. Table 4 shows the results secured. The iodine value and Reichert-Meissl number increased 4.24 and 0.72 respectively, whereas, the saponification value decreased 2.3 and the hardness declined 264 grams. Except for the Reichert-Meissl these alterations from the basal periods are in the same direction as those shown in Table 3 for oats and corn feeding. The higher iodine value obtained on the basal ration in Table 4, as compared with Table 3, may be attributed to advance in lactation. The body of the butters was normal in all cases.

b. *Influence of feeding corn oil at higher levels*—Table 4 also shows results following the feeding of one pound of corn oil to Group III and IV and 0.8 pound of corn oil to cow 151. The saponification value declined 0.8 for cow 151 and 7.7 for Group III. The iodine value increased 3.28 for cow 151 and 10.50 for Group III. Changes in Reichert-Meissl number were not consistent and in melting point were insignificant. An unmistakable decrease in hardness of the fat resulted, this decrease being 281 grams for cow 151, 424 grams for Group IV, and 371 grams for Group III.

Thiocyanogen values were run on the butterfat from cow 151 in the basal and corn oil feeding periods using the method of Kaufman (10) as modified by Zeleny and Bailey (11). Using the difference between the iodine value and the thiocyanogen value as a measure of fatty acids less saturated than oleic it was found that the difference for the corn oil feeding period was only 0.38 greater than for the mean difference of the two basal periods, being 3.02 for the latter and 3.40 for the former.

c. *Influence of feeding linseed oil*—Linseed oil was fed in quantities from 0.8 to 1.25 pounds daily. Table 5 gives the results. Group V showed the greatest changes in iodine value, but the decrease in hardness was

TABLE 5

*The effect of feeding linseed oil and cottonseed oil on the chemical constants and hardness of butterfat*

Group	Amount of oil fed lbs.	Chemical constants			Hardness, grams
		Saponification value	Iodine value, per cent	Reichert-Meissl number	
	Linseed oil				
Cow 419	0.0	231.9	34.00	28.81	1138
	0.9	227.2	42.21	27.63	922
Difference		- 4.7	+ 8.21	- 0.18	- 216
Cow 151	0.0	233.7	29.29	29.20	1885
	0.8	229.7	41.17	30.11	1047
Difference		- 4.0	+ 11.88	+ 0.91	- 838
V	0.0	227.7	38.17	26.33	1126
	1.25	219.1	54.28	24.34	771
Difference		- 8.6	+ 16.11	- 1.99	- 355
VI	0.0	226.8	42.45	25.73	725
	1.25	218.6	50.85	22.87	685
Difference		- 8.2	+ 8.40	- 2.92	- 40
	Cottonseed oil				
III	0.0	236.0	30.47	32.45	1502
	1.0	228.9	38.35	33.20	1199
Difference		- 7.1	+ 7.88	- 0.25	- 303
IV	0.0	235.1	30.22	30.84	1502
	1.0	229.8	37.60	30.76	1677
Difference		- 5.3	+ 7.38	- 0.12	+ 175
Cow 151	0.0	231.5	33.68	28.74	1330
	0.8	231.8	33.12	28.43	1273
Difference		+ 0.3	- 0.56	- 0.31	- 57

about midway between the greatest and the least observed in the trial. The increase in iodine value was least for Group VI, although the same amount of oil was fed as in the case of Group V. Hardness for Group VI declined only 40 grams; it is evident that the decline in hardness which might have been expected as the result of the higher iodine value was very nearly offset by the decline in Reichert-Meissl number. Cow 151 showed an increase of 11.88 in iodine value of her butterfat and the hardness dropped 838 grams, whereas cow 419 showed an increase of 8.21 in iodine value accompanied by a decrease of 216 grams in hardness. In the one case (cow 151) both the unsaturated and the volatile fatty acids increased, while in the other the increase in unsaturation was offset, in part, by a decrease in volatile fatty acids. The saponification values in general followed the changes in Reichert-Meissl numbers, as would be expected.

The mean difference between iodine and thiocyanogen values for the basal ration periods preceding and following the linseed oil feeding was 3.53. In the oil feeding period it was 4.98, showing that linseed oil causes a definite rise in the fatty acids less saturated than oleic acid in butterfat.

d. *Influence of feeding cottenseed oil*—Table 5 also gives the effects of feeding cottenseed oil. Comparable results were obtained for Groups III and IV except for hardness, which increased 175 grams for Group IV and decreased 303 grams for Group III. Cow 151 did not show changes in fat constants of as great a magnitude as either Group III or IV, her butterfat remaining very constant in hardness. It is evident from the results for Groups III and IV that the effect of cottonseed oil feeding on the hardness of the butterfat cannot be predicted from the change in iodine value. When cow 151 was fed cottonseed oil, the butter had a gummy body that was unmistakably different from that produced during corn oil or linseed oil feeding.

The cottonseed oil had very little effect in increasing the fatty acids less saturated than oleic acid, judging from the difference between the iodine and thiocyanogen values of the butterfat of cow 151. In the period preceding the oil feeding the difference was 3.05 and in the oil feeding period it was 3.15.

e. *Influence of feeding tallow*—The results of adding one pound of tallow to the basal ration are given in Table 6. It is evident that the tallow depressed the saponification value, with but little change in iodine value or Reichert-Meissl number. This points to an increase in the non-volatile saturated fatty acids since the saponification value decreased without an alteration in the iodine value. The melting point and hardness were increased, but not to the same degree in each case.

f. *Influence of feeding butterfat*—The oils fed in the preceding experiments were of a very different composition than butterfat. These oils had a low saponification value, high iodine value, and no volatile fatty acids. Tallow did not have such a high iodine value, but the saponification value was low. Butterfat was fed in an attempt to determine the effect of feeding a fat containing all the fatty acids of the resulting product.

It will be observed in Table 6 that feeding rendered butterfat brought about some very significant changes in the properties of the butterfat produced. The iodine value decreased 1.69 for Group IV and increased 0.46 for Group III. The average decline in saponification value was 3.0 with a decrease in Reichert-Meissl number of 0.62. Melting point and hardness were augmented most. These changes are much greater than those observed for tallow feeding. The small drop in saponification value indicates an increase in saturated acids, since the iodine value did not increase appreciably. The changes in fat constants fail to explain the differences observed in melting point or hardness of the butterfats obtained.

TABLE 6

*The effect of feeding tallow, butterfat and coconut fat on the chemical and physical constants of butterfat*

Group	Amount fat fed lbs.	Chemical constants			Physical constants	
		Saponi- fication value	Iodine value per cent	Reichert- Meissl number	Melting point ° C.	Hardness grams
	Tallow					
III	0.0	237.5	31.44	29.35	33.50	1430
	1.0	229.8	32.61	29.59	34.35	1651
Difference		- 7.7	+ 1.17	+ 0.24	+ 0.85	+ 221
IV	0.0	235.2	30.60	30.60	33.00	1730
	1.0	230.2	30.71	30.71	34.35	1745
Difference		- 5.0	+ 0.11	+ 0.11	+ 1.35	+ 15
	Butterfat					
III	0.0	233.4	28.90	28.83	33.50	1815
	1.0	230.0	29.36	28.63	35.25	2246
Difference		- 3.4	+ 0.46	+ 0.20	+ 1.75	+ 431
IV	0.0	234.0	29.47	30.36	32.95	1675
	1.0	231.5	27.78	29.15	35.45	2389
Difference		- 2.5	- 1.69	- 1.21	+ 2.50	+ 714
	Coconut fat					
151	0.0	233.4	29.25	29.00		1791
	0.8	238.4	24.65	29.22		2210
Difference		+ 5.0	- 4.60	+ 0.22		+ 419
419	0.0	232.8	32.45	29.37		1212
	1.0	235.9	28.96	28.96		1743
Difference		+ 3.1	- 3.49	- 0.41		+ 531

The body of these butters was firm but not hard or brittle.

g. *Influence of feeding coconut fat*—The preceding experiments have shown the effects on the fat constants produced by feeding oils of fats having a wide range of iodine values. It was therefore considered important to include in further experiments a highly saturated fat. Coconut fat contains only 6–10 per cent oleic acid and approximately one per cent of linoleic acid. It also has a higher saponification value than the oils or fats fed in the foregoing experiments. The data presented in Table 6 give the individual results following the feeding of coconut fat. The iodine value was depressed 4.6 for cow 151 and 3.49 for cow 419, the saponification value increased 5.0 for cow 151 and 3.1 for cow 419, and the hardness of the butterfat increased 419 grams for cow 151 and 531 grams for cow 419. These butters were very hard and brittle, especially that from cow 151, which was somewhat crumbly. The structure of the butterfat in these butters appeared to be almost crystalline when they broke at temperatures of 54 degrees F.

Thiocyanogen values were also determined in connection with the coconut fat feeding. The average differences between iodine values and thio-

cyanogen values for the two cows were as follows: For the basal periods, 2.87; for the coconut fat feeding, 2.41. The results indicate some decrease in acids less saturated than oleic due to feeding coconut fat.

h. *Influence of feeding cane sugar*—In the experiments so far reported the effects of various oils and fats on the composition and properties of butterfat were studied through the addition of the oils and fats to the basal ration. This also involved an increase in digestible nutrients of the ration in a special form. In order to determine the effects of the addition of the same amount of nutrients in other forms, an experiment was conducted in which cane sugar was added to the basal ration. It was hoped that this experiment would also assist in interpreting the data reported in the literature relative to the relation of easily fermented carbohydrates in food to the formation of the volatile fatty acids in butterfat. The results in Table 7 show an average increase in saponification value of 2.0, in Reichert-Meissl number of 0.92, and in hardness 146 grams. The iodine value changed but slightly, decreasing 0.64. The increase in hardness corresponds with the slight decrease in iodine value.

TABLE 7

*The effect of feeding cane sugar on the chemical constants and hardness of butterfat*

	Amount fed lbs.	Chemical constants			Physical constants	
		Saponification value	Iodine value per cent	Reichert-Meissl number	Melting point ° C.	Hardness grams
Group III	0.00	233.4	28.03	28.33	33.20	1611
	2.25	237.0	27.10	30.66	33.10	1735
	Difference	+ 3.6	- 0.93	+ 2.13	- 0.10	+ 124
Group IV	0.00	236.2	27.87	30.99	33.10	1798
	2.25	236.6	27.42	30.70	33.40	1947
	Difference	+ 0.4	- 0.35	- 0.29	+ 0.30	+ 149

*The Effect of Including Pasture in the Ration*

Analyses were made of the butterfat from the University herd previous to turning onto grass and for two weekly periods thereafter. This change is shown in Table 8. The effect was a decrease in saponification and Reichert-Meissl numbers of 2.2 and 0.43 respectively with an increase in iodine value of 3.17. No difference was found in the hardness of the butterfat.

Two cows, 151 and 419, were removed from pasture during the summer in order to throw further light on the effect of pasture on the character of the butterfat. Table 8 shows the results from these two cows. The average effects were as follows: the saponification value increased 2.15, the Reichert-Meissl number decreased 0.20, and the hardness increased 372 with a decrease in iodine value of 3.77. The changes observed in this

TABLE 8

*The effect of including pasture in the ration on the chemical constants and hardness of butterfat*

Sample	Ration	Chemical constants			Hardness grams
		Saponi- fication value	Iodine value per cent	Reichert- Meissl number	
Station herd	Station ration	229.7	35.25	29.08	952
	Pasture and herd ration	226.5	38.42	28.65	951
	Difference	- 2.2	+ 3.17	- 0.43	- 1
419	Pasture and herd ration	224.3	43.18	27.40	606
	Basal	226.6	40.18	27.85	657
	Difference	+ 2.3	- 3.00	+ 0.45	+ 51
151	Pasture and herd ration	231.8	34.65	29.06	990
	Basal	233.8	30.11	28.30	1684
	Difference	+ 2.0	- 4.55	- 0.86	+ 694

experiment, which may be attributed to grass, are much less than those reported in the literature.

#### *The Composition and Properties of Six Commercial Samples of Butter*

The experiments recorded in the preceding pages have shown that certain factors produced butterfat of a low iodine value exhibiting hard characteristics. It seemed of interest to compare these results with those from samples of butter obtained from regions where similar body defects have been reported. Normal samples were also obtained for comparison. Five of these samples were made available through the courtesy of Dr. G. H. Wilster of the Oregon Agricultural Experiment Station, the other by Mr. Al Forte from the Mandan Creamery, North Dakota. The chemical constants and history of the samples are given in Table 9.

The data reveal considerable difference in the hardness of the butterfats with only a slight range in the iodine value. The hard butters (1, 2, and 5) were from sections in which Jerseys are the predominating breed and alfalfa hay is fed in large quantities. The grain ration is made up largely of barley and millrun, a small quantity of linseed meal being included in the concentrate for part of the herds. Sample 3 apparently was influenced by the fall pasture; in addition, the mixed grains contained a considerable quantity of oats. It is evident from the hardness studies and the comments on the butters that those samples associated with alfalfa feeding are most abnormal. However, barley was included in the grain ration for the herds from which the samples were produced. Furthermore, Jerseys were the predominating breed. Breed exerts a definite influence on hardness of butter as shown by Coulter and Hill (8).

TABLE 9

*The chemical constants, hardness, body criticisms and history of six commercial samples of butter*

Sample number	Body criticism	Chemical constants			Hardness grams
		Saponification value	Iodine value per cent	Reichert-Meissl number	
1	Slightly crumbly and brittle	231.5	32.48	26.93	1951
2	Sticky	230.1	31.10	26.44	1860
3	Normal	226.9	33.43	27.44	1261
4	Normal but firm	230.3	31.89	29.95	1471
5	Slightly crumbly	231.8	30.69	27.33	2214
6	Normal but firm	228.9	32.58	27.38	1423

History of samples				
Sample number	Place made	Breed of cows		Feeds
		Predominating	Others	
1	Klamath County, Oregon	Jersey	Red cows	Alfalfa hay, cull potatoes, barley, millrun, and linseed meal.
2	La Grande, Ore.	Jersey	Shortborns	Alfalfa, corn, silage, barley, millrun, and linseed meal.
3	Gray's River, Washington	Jersey and Guernsey		Graas hay, mixed grain, fall pasture.
4	Corvallis, Ore.	Jersey		Oats and vetch hay, clover hay, mixed silage, kale, millrun chief concentrate.
5	Redmond, Ore.	Jersey		Alfalfa hay, barley, millrun, <sup>1</sup> and linseed meal.
6	Mandan, N. Dak.	Mixed cows all breeds		Mixed hay (chiefly prairie) and corn.

<sup>1</sup> Millrun is made up of the by-products of flour manufacture consisting of bran, middlings, and in some cases ground screenings.

#### DISCUSSION

Changing from the Experiment Station herd ration, consisting of the herd grain ration, alfalfa hay, and corn silage, to the experimental ration and timothy hay or alfalfa hay, exerted a very profound influence on the characteristics of the butterfat. The averages of the effects were: The iodine value declined 4.49, the Reichert-Meissl number declined 1.01, but the melting point and hardness increased 1.82 degrees C. and 748 grams, respectively. The only change of any significance in the nutrients supplied was a decrease of 0.198 pounds of fat daily. This decrease in amount of fat in the ration was apparently responsible for producing the hard butters observed in both groups.

The fat constants, hardness, and melting point of butter obtained when using timothy hay, alfalfa hay, and beet pulp were not significantly different. It is evident that the fat from each of these feeds exerts an equal effect on the composition of butterfat when fed in combination with a low fat grain mixture. These feeds produced a hard butter not conforming to the characteristics usually associated with superior butter.

Increasing the alfalfa in the ration to such an extent that 85 per cent of the total digestible nutrients were supplied from it brought about rather marked changes in the butterfat of one Holstein cow used in the test. The chief effect was an increase of 4.36 in the iodine value and a decrease of 252 grams in the hardness of the butterfat. The Reichert-Meissl value decreased 1.64. These changes in iodine value conform closely to those reported by Richardson and Abbot (9) for butterfat from cows restricted to alfalfa feeding. However, these workers report a "sticky" body for these butters which was not found in the experiment reported here.

Including pasture in the herd ration resulted in an increase of 3.17 in the iodine value without any effect on the hardness of the butterfat. With two individual cows the reverse procedure, *i.e.*, removal of the cows from pasture to the basal ration, decreased the iodine value and increased the hardness of the butterfat, especially when the decrease in iodine value was accompanied by a decrease in Reichert-Meissl number.

The results with the cereals demonstrate that the effect of corn, oats, and barley are due to the character and amount of the fat they contain. Table 3 shows conclusively that barley feeding resulted in the production of a hard butter, whereas corn and oats produce butters with a desirable body and firmness. Corn oil, when added to the barley-bran ration in quantities equivalent to the difference between the oil content of the barley plus bran and the corn plus bran rations, brought about results very similar to those produced when corn was fed. These experiments were undoubtedly influenced somewhat by outside factors not under control; however, the results are sufficiently consistent to justify the above conclusion.

The literature appears to show that the iodine value of the oil in a feed or ration will be directly responsible for the hardness of butterfat. This apparently holds true insofar as certain oils are concerned, but does not hold true in all cases. It is true that the iodine value of the ration fat largely determines the iodine value of the butterfat. For example, linseed oil increased the iodine value most with corn oil and cottonseed oil following rather closely. Both tallow and butterfat being about equally unsaturated had but slight influence on the iodine value of the butterfat. Coconut oil being very highly saturated produced a butterfat having a lower iodine value and higher saponification value than did the basal ration. Considering that the above oils were added to a ration already supplying sufficient total digestible nutrients to satisfy the requirement, it

is very evident that these oils modified the fat forming processes already going on in the animal body.

However, the oils and fats in feed do not influence the hardness of butterfat to the same degree that they affect the iodine value. Corn oil influenced the hardness equally as much as the linseed oil. Examination of the butter produced during the feeding of linseed oil revealed a somewhat firmer body than that produced during corn oil feeding. The butter from corn oil feeding tended to be somewhat softer and mushy. Cottonseed oil, on the other hand, increased the iodine value but did not appreciably decrease the hardness except with one group of cows; furthermore, the body of the butter was gummy and sticky and did not melt easily when tasted. Tallow did not appreciably alter the composition of the fat or the hardness of the butterfat. Butterfat feeding likewise did not alter the iodine value or Reichert-Meissl number to any marked extent; however, it did increase very markedly the melting point and the hardness of the fat. The butters in these cases were tough but not brittle, indicating further change in the structure of the triglycerides.

Recent experiments by Schoenheimer (12) may explain the failure of the volatile fatty acids fed in the butterfat feeding periods to appear in the milk fat. Using deuterio-fatty acids to trace their fate after ingestion, he reports that butyric and caproic acids are completely oxidized (by mice) and only higher fat acids are deposited.

Coconut fat unmistakably decreased the iodine value of the butterfat, increased the saponification value, and markedly increased the hardness of the butterfat. The change observed was dependent on the initial properties of the butterfat; since cow 419 was producing a relatively normal fat on the basal ration, the addition of coconut fat did not produce a butter exhibiting a body of a firmer character than might be expected from feeding a low fat diet. In contrast, cow 151, already producing a relatively hard butter, produced a very hard, brittle butter, approaching crumbliness, following the coconut oil feeding. This butter was nearly the hardest produced during the experiment. The fats had iodine values very similar to those observed in the roughage feeding tests; however, the other characteristics of the fat were considerably different.

The feeding of sugar had no discernible effect on the fat constants. The hardness was increased slightly but not sufficiently to be of any real importance, thus further supporting the assumption that the fat is a more important factor influencing butterfat composition than the energy supplied.

The discussion concerning the hardness of butterfat and the iodine value tends to emphasize certain specific effects for feeds other than those exerted on the iodine value. Generally speaking it is possible to deduce from Coulter and Hill (8) that there is a highly significant relationship between iodine value and hardness of butterfat. The index of correlation was 0.85 with less than one chance in 100 that this value could come from chance.

Cottonseed oil feeding gave hardness values entirely out of line with the iodine value, especially in the group feeding tests.

Hilditch and Sleightholme (13) and Banks and Hilditch (14) have discussed the importance of the saturated triglycerides in feed imparting to animal fats their characteristic properties such as melting point and consistency. In all the samples reported by these workers the fully saturated portions of the butterfat were very similar in composition. Following the feeding of oils the changes of most importance occurred in the percentage of non-fully-saturated glycerides. When considering these findings in relation to the results obtained in the present study it appears that an increase in non-fully-saturated glycerides, in addition to an increase in the quantity of unsaturated acids present in the portion previously non-fully-saturated, no doubt played an important part in the changes observed in hardness. The decrease in the slope of the curve shown by Coulter and Hill (8) may be explained by a more complete unsaturation of the non-fully-saturated triglycerides. In this case the fully-saturated glycerides could exert a more constant effect in retaining the consistency of the butterfat.

#### CONCLUSIONS

1. When barley constitutes 35-50 per cent of the digestible nutrients of a low fat ration containing alfalfa or timothy hay, fed to dairy cows, hard butterfat with a low iodine value is produced.

2. When oats or corn are substituted for 35-50 per cent of the digestible nutrients of a low fat ration, containing alfalfa hay, fed to dairy cows, the physical characteristics of the butter produced are satisfactory from the market standpoint, and the chemical characteristics of the fat are more or less specific for the type of ration fed.

3. When alfalfa hay, timothy hay or beet pulp are fed with a low fat grain ration to dairy cows, they exert a similar effect on the composition and physical properties of butterfat.

4. When oils or fats are fed to dairy cows the resulting butterfat assumes some of the chemical characteristics of the fat or oil fed.

5. When 0.6 pounds or more of linseed oil is fed daily to dairy cows the butterfat produced shows a significant increase in the content of fatty acids less saturated than oleic acid, as judged by the increase in difference between the iodine and thiocyanogen values.

The late Doctor C. H. Eckles suggested this study. His help and criticism in planning and carrying out the investigation is gratefully acknowledged.

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# SOME CAUSES FOR THE DETERIORATION IN 10 DAYS AT 15.5° C. OF SALTED BUTTER MADE FROM SOUR CREAM<sup>1</sup>

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Much research work (4, 5, 9, 10, 11) has been done on the problem of keeping quality of butter in commercial cold storage; however, less consideration (6, 13) has been given to keeping quality of the butter while it is in the various trade channels and in the home of the consumer where it may be held for periods of time at temperatures far too high for satisfactory butter storage. During this time, when at relatively high temperatures, some butter preserves its flavor while in other butter the flavor deteriorates. In order to determine some of the causes of these flavor changes, this study was undertaken.

An explanation of the possible causes of deterioration in flavor have been bacteria, yeasts, molds, the inherent enzymes of the milk, and chemical action taking place within the constituents of butter.

For this study a number of samples of typical centralizer butter made in Indiana and neighboring states were available. In addition to the microbiological analyses, each sample was scored when obtained and given a keeping quality test which consisted of holding the butter for 10 days at 15.5° C. and then rescored. The scoring was done by Dr. B. E. Horrall and the scores were then checked by the authors individually and average scores recorded. The butter was examined bacteriologically, after incubation, in an effort to determine some of the factors that were involved in its deterioration.

## THE KEEPING QUALITY OF SALTED BUTTER MADE FROM SOUR CREAM

In this study 504 samples were examined. The butter was manufactured in a nine-month period starting September, 1936 and the number of samples received each month was approximately the same and from the same creameries. The drop in score in samples of butter during the 10 day holding period at 15.5° C. is shown in Table 1.

It may be assumed that butter which does not decrease in score more than one point will stand up under the conditions of refrigeration used by distributors and consumers. As shown in Table 1, 25 per cent of the samples

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<sup>1</sup> The data in this paper was taken in part from a thesis presented by J. C. Flake in partial fulfillment of the requirement for the Master of Science degree, and is published with the approval of the Director of the Agricultural Experiment Station.

TABLE 1

*The keeping quality of salted butter made from sour cream as indicated by change in score after 10 days at 15.5° C.*

	Drop in score in points				
	0	0.5-1	1.5-2	2.5-3	3.5 or more
Number of Samples .....	163	214	73	39	15
Percentage of Samples ..	32	42	14	8	3

decreased in score more than one point and 11 per cent decreased 2.5 points or more in score.

TABLE 2

*The drop in score of butter after holding for 10 days at 15.5° C. according to the original score of the same butter*

	Original Score			
	91 or more	90.5-90	89.5-89	88.5 or less
Number of Samples .....	31	157	255	63
Average Drop in Score ....	2.0	0.86	0.60	0.68
Per Cent not Dropping in Score .....	0	27	38	37

In Table 2 the data are presented to show the influence of the original score of the butter upon its keeping quality as measured by the method used. The bulk of the samples examined had original scores between 90.5 and 89. However, in the butter scoring 91 or more, the deterioration was so consistent that the results are significant in that no sample was found which did not decrease in score and this average decrease was 2 points. The decrease in score of the butter scoring 90.5 to 90 was 0.86 points and 27 per

TABLE 3

*Relation of the month of manufacture to the drop in score of butter*

Month 1936-37	No. of Samples	0-5	Percentage of samples decreasing in score	
			1-1.5 points	2 or more points
September .....	54	30	48	22
October .....	59	27	54	19
November .....	61	44	41	15
December .....	60	40	42	
January .....	57	30	47	23
February .....	53	37	47	16
March .....	50	48	38	14
April .....	61	69	23	8
May .....	52	47	38	15

cent of the samples retained their original score. Butter which scored below 88.5 showed approximately the same drop in score and with the same percentage of samples decreasing in score as did butter scoring 89.5 to 89.

In Table 3 are presented data showing the relation of the month in which the butter was manufactured to decrease in score in 10 days at 15.5° C.

The butter manufactured during the colder months of the year showed no significantly better keeping quality than butter made in the fall or spring. In January, butter of the poorest keeping quality was manufactured, while in April the butter of the best keeping quality was manufactured of the months studied. However, in May, the keeping quality decreased approaching the median for the period.

#### THE INFLUENCE OF THE MICROBIOLOGICAL CONTENT OF THE BUTTER UPON ITS KEEPING QUALITY

In that butter consists of 80 per cent fat, which is an inert material to many micro-organisms, it presents a microbiological problem entirely different from that of other dairy products. This is further emphasized by the fact that butter is an emulsion of water in fat, while in milk and most other dairy products the water is the continuous phase.

It was deemed advisable to study the general microscopic picture of the butter flora to determine whether or not micro-organisms were the cause of the decrease in score. This was done by preparing a sample of centrifugally separated serum using the method developed by Hammer and Nelson (3) and staining with the technique of Newman (7).

The examination of 103 samples of butter which had dropped from zero to four points in score during the holding period revealed that there was a limited relation between the microscopic picture and the decrease in score. In general, it was found that large numbers of rod-shaped organisms were found in butter having poor keeping quality. These results are similar to those reported by Nelson (6). No relation was found to exist between the decrease in score and the numbers or arrangement of cocci contained in the smear of butter serum.

Previous workers (2, 11, 12) have endeavored to determine the influence of proteolytic and lipolytic organisms upon the keeping quality of butter by enumerating the numbers of these organisms in freshly manufactured butter. Since there are a number of factors other than time and temperature of incubation which affect the growth of micro-organisms in butter, a study was made to determine the numbers of proteolytic organisms in the butter at the end of the holding period of ten days at 15.5° C.

At the conclusion of the holding period, the samples of butter were plated on tryptone-skim milk agar for the purpose of detecting proteolytic organisms. The composition of the medium used, as modified from the casein containing medium suggested by the American Dairy Science Association

Sub-Committee on Microbiological Methods of Examining Butter (8), was as follows: 0.5 per cent tryptone, Difco; 1.5 per cent agar agar; 5.0 per cent skim milk, added just before pouring plates. The final pH was 6.8 to 7.2. Incubation was for five days at 21° C. Proteolytic colonies were judged by their appearance and when in doubt the plates were flooded with five per cent tannic acid.

The results from 101 samples selected at random from the 504 samples are shown in Table 4, with the counts tabulated according to drop in score of the butter. The samples which dropped less than one and one-half points in score had comparatively low logarithmic average proteolytic counts. The averages for the samples which dropped two to two and one-half points are

TABLE 4

*Relation of proteolytic bacterial count to drop in score of butter held for ten days at 15.5° F.*

	Drop in Score in Points							
	0	1	1.5	2	2.5	3	3.5	4 or more
Number of Samples .....	22	9	18	22	8	12	4	6
Logarithmic Average of Proteolytic Counts .....	9,250	2,350	5,000	31,000	23,000	7,500	4,000	32,000
Proteolytic Bacterial Counts of 100,000 or more—per cent .....	4.5	11.0	5.6	36.4	50.0	16.7	25.0	50.0

much higher, as is true for those which dropped four or more points. However, the samples which dropped three to three and one-half points show low proteolytic counts, being similar to the samples which had only a slight drop in score. The reason for these low counts is difficult to explain, especially in view of the limited number of samples. The organisms may have died off while the butter was being incubated because of limited food or because of the presence of poisonous products of metabolic activity. The deterioration may have been more concerned with chemical than with biological causes. Another possibility is that the right balance of associative action was present in order to cause marked deterioration although the actual proteolytic counts were low.

Table 4 is even more significant when the distribution of the counts of 100,000 or more proteolytic organisms per ml. are examined. Further tabulation shows that, of the samples dropping less than two points, only 6.1 per cent had counts of 100,000 or more, while of the samples which dropped two or more points, 34.6 per cent had counts falling within this range.

It is believed that proteolytic organisms are an important factor in the development of putrid flavors in butter. It was found that the samples which developed this flavor during the holding period had higher proteolytic counts than did the samples which developed other off flavors. Thirty-three and six-tenths per cent of the samples which developed putrid flavors had proteolytic counts of 100,000 or more. Of those which developed rancid flavors, old cream flavors, and those which did not change in score, 16.6, 11.1, and 4.5 per cent respectively, had proteolytic counts of 100,000 or more.

The samples were also plated at the end of the ten day holding period for the enumeration of lipolytic micro-organisms. Tributyrin agar and nile blue sulfate agar were used. Incubation was for five days at 21° C.

The tributyrin medium, as modified from that suggested by Anderson (1), consisted of Standard Nutrient Agar base, plus 0.1 per cent sodium taurocholate, plus 1.0 per cent tributyrin. The final reaction was pH 6.8 to 7.2.

The nile blue sulfate medium, as modified from the one suggested by the American Dairy Science Association Sub-committee on the Microbiological Methods of Examining Butter (8), consisted of 0.5 per cent tryptone (Difco), 1.5 per cent agar agar, 0.5 per cent skim milk, 5.0 per cent of a 0.1 per cent nile blue sulfate solution, and 10.0 per cent of a butterfat emulsion containing 4.0 per cent butterfat and 0.5 per cent agar agar. The sterile fat emulsion and skim milk were added just before pouring the plates. The final reaction of the medium was pH 6.8 to 7.2.

The results of plating these samples of butter on the two media for the detection of lipolytic bacteria are given in Tables 5 and 6.

TABLE 5

*Relation of lipolytic count on tributyrin medium to drop in score of butter held for ten days at 15.5° C.—Total of 63 samples*

Drop in Score	0	1	1.5	2	2.5	3	3.5	4 or more
No. of Samples	16	6	9	10	5	9	3	5
Log. Av. of Counts	120,000	150,000	130,000	890,000	480,000	590,000	1,350,000	680,000

Table 5 shows a very marked relation between high lipolytic counts on tributyrin medium and poor keeping quality during the ten day holding

TABLE 6

*Relation of lipolytic bacterial count on nile blue sulfate medium to drop in score of butter held for ten days at 15.5° C.—Total of 101 samples*

Drop in Score	0	1	1.5	2	2.5	3	3.5	4 or more
No. of Samples	22	9	17	22	9	12	4	
Log. Av. of Counts	7,400	9,200	21,500	117,500	17,000	38,000	26,000	2,050

period. Further tabulation gives a logarithmic average of 127,000 per ml. for the samples which dropped less than two points, while those which dropped two or more points had a logarithmic average count of 700,000 per ml. of butter.

Table 6 shows a marked tendency for high lipolytic counts on nile blue sulfate medium to be associated with poor keeping quality, except in the case of those samples which decreased 4 or more points in score. There is, however, a less direct relation between lipolytic count and keeping quality when this medium is used than when the tributyrin media was used.

#### SUMMARY AND CONCLUSIONS

The data presented show that salted butter made from sour cream presents a problem as far as keeping quality at 15.5° C. is concerned. Twenty-five per cent of the 504 samples received from September to May dropped at least one and one-half points in score. This deterioration was found among the samples submitted by a large number of different creameries.

The samples which had an original score of 89 to 89.5 points had better keeping quality than did higher scoring butter and slightly better keeping quality than did the butter which scored less than 89. The month in which the butter was made did not significantly influence its deterioration.

A limited relation between the microscopic picture and keeping quality of the butter was found. Large numbers of rod-shaped organisms were, in general, associated with poor keeping quality. There was no relation between keeping quality and numbers or arrangement of cocci.

There was a marked relation between high proteolytic counts on tryptone-skim milk agar and poor keeping quality.

A marked relation was found between high lipolytic counts on tributyrin medium and poor keeping quality. This relationship was less marked when nile blue sulfate medium was used.

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# THE CORRELATION BETWEEN ORGANISMS FOUND MICROSCOPICALLY IN BUTTER SERUM AND THE GRADE OF CREAM FROM WHICH THE BUTTER WAS MADE

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Quality in butter is becoming a more important issue to the industry each year. Since butter quality depends on the grade of cream, the cream usually has been graded in accordance with the kind of butter it will make. Grading is accomplished by the senses of taste and smell. In this study an attempt was made to determine the grade of cream by a microscopic examination of the organisms in the butter serum. This also included studies on varied lengths of storing butter, effects of culture, and a possible relationship between specific flavor defects and certain micro-organisms. Not all deterioration of cream is bacteriological so the grade may be lower than the organisms and their action would indicate.

## PREVIOUS INVESTIGATIONS

Nelson (1) studied microscopic slides of butter with the view of predicting its keeping quality during storage for 7 days at 21° C. Clumps of well stained thin rods were generally a sure sign that deterioration would take place, especially in unsalted butter. The keeping quality was correctly predicted on 96.4 per cent of the commercial salted samples, 79.6 per cent of the unsalted and 84.9 per cent of the exhibition butter.

Nelson and Hammer (2) found that butter culture streptococci generally developed little or not at all in salted butter held at a favorable growth temperature. Organisms other than streptococci sometimes showed growth, this growth depending on the species present. In unsalted butter both streptococci and organisms other than streptococci developed at a favorable temperature.

Macy, Coulter and Combs (3) obtained decreased counts on salted butter held for 30 days; molds 66.7 per cent, yeasts 80 per cent and 73.3 per cent for bacteria. These decreases did not follow a consistent pattern.

## PROCEDURE

Cream of all grades was procured and scored by two experienced judges according to the grade of butter it would make. Sweet and clean cream which should produce butter scoring 93 or above was graded "excellent," clean and sour cream scoring 91½ to 92½ was graded "good," and cream graded "fair," which scored 90 to 91 was sour with some off flavor. Cream

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scoring below 90 was graded "poor." After scoring a microscopic slide was prepared from each sample. The cream was pasteurized at 68 to 71° C., held for 30 minutes, cooled to 7° C., held over night at 4° C., churned in a small experimental churn, buttermilk drained, butter washed, salted and worked. A sample of butter was then taken in 4 ounce bottles and held at 4° C. until slides were prepared of it.

Cream slides were prepared by the Fay (4) method. It consisted of 0.1 ml. of cream, one drop of sterile water, and one drop of Mayer's egg-glycerine mixture spread on one-half the surface of a slide. This was dried, dipped in alcohol for 20 seconds, dried and immersed in xylene for two minutes, dried and stained for one minute in methylene blue. Butter slides were prepared according to Nelson and Hammer (5). Melted butter was centrifuged in a separatory funnel and the non-fatty layer, serum, drained. One hundredth of a ml. was spread over 1 square centimeter, dried, stained with Newman stain (6), washed, dried, and restained with methylene blue.

#### RESULTS

One hundred and thirty samples were studied in the preliminary work to acquire a knowledge of the microscopic appearance of butter slides for the grades of cream "excellent," "good," "fair," and "poor." The following appearances of butter slides were correlated with the various grades of cream: (1) Butter from "excellent" grade cream showed a few well stained micro-organisms. Those present were cocci singly, in pairs, or chains. The use of culture increased the number of organisms present. No rods and only a very few yeasts were ever seen. (2) Slides of butter from "good" cream had a large number of well stained micro-organisms most of which were cocci singly, in pairs, or chains. A very few partly decomposed cells, occasionally occurred and a very few yeasts or molds. (3) "Fair" grade cream gave butter whose slides had an exceedingly large number of well stained micro-organisms. Most were cocci singly, in pairs, and in chains. The slides frequently showed many poorly stained and partly decomposed cells, a few rods, yeasts, and molds. (4) Slides from butter made of "poor" grade cream had a large to enormously large number of organisms, a majority of these were stained poorly and partly decomposed. Often slender rods existed; many times yeasts and molds were present.

The results of the study were grouped according to the grade of cream. On table 1 are tabulated the studies of butter made from cream scoring "excellent." A total of 143 different samples of butter was examined, of which 33 were stored for 1-3 days before preparing the slides. The grade was determined correctly by the microscopic examination on 90.9 per cent of the samples. For the 7-day holding, 95.4 per cent of 22 samples were correctly graded. Of the 56 samples held 14 days, 89.3 per cent were graded rightly. The fourth group consisted of 32 samples held 30 days, and 93.7

per cent were graded accurately. The average of the four holding periods was 91.6 per cent correct out of 143 samples. The number of samples of other grades incorrectly called "excellent" was small; 9.6 per cent of 125 in the "good" cream class, 1.8 per cent of 159 in the "fair" class and 4.2 per cent of the 97 "poor" cream samples. This is a total of 4.9 per cent mis-graded as "excellent" from 37 samples.

TABLE 1

*Butter churned from "excellent" cream scoring 93 or above*

	Storage Period			
	1-3 days	7 days	14 days	30 days
Total Samples ...	33	22	56	32
Number graded right ...	30	21	50	30
Number graded wrong ...	3	1	6	2
Number missed 1 grade	3	1	1	1
Number missed 2 grades	0	0	0	1
Number missed 3 grades	0	0	5	0
Per cent graded right	90.9	95.4	89.3	93.7

Table 2 contains the information on butter churned from "good" cream. Thirty-three samples were held 1 to 3 days and 63.6 per cent were graded correctly, 24 samples held for 7 days and 66.6 per cent graded correctly, 37 samples held 14 days of which 43.2 per cent were graded correctly. In the group held 30 days, 64.5 per cent were graded correctly from 31 samples. The total of the four holding periods amounts to a percentage of 58.4 right from 125 samples. The percentage of other grades incorrectly called "good" were: "excellent" 4.1, "fair" 27.6, and "poor" 19.5, giving a 17.2 per cent total on the three grades.

The tabulations for "fair" cream was recorded on table 3. The group stored for 1 to 3 days contained 57 samples and only 43.9 per cent were

TABLE 2

*Butter churned from "good" cream scoring 91½ to 92½*

	Storage Period			
	1-3 days	7 days	14 days	30 days
Total Samples .....	33	24	37	31
Number graded right .....	21	16	16	20
Number graded wrong .....	12	8	21	11
Number missed 1 grade too high .....	1	2	4	5
Number missed 1 grade too low .....	8	3	5	5
Number missed 2 grades too low .....	3	3	12	1
Per cent graded right .....	63.6	66.6	43.2	64.5

accurately graded. In 7 day lots 60.0 per cent were ascertained rightly from 20 samples and 40.0 per cent of the 40 samples in storage 14 days. Forty-two samples were stored in the 30 day lot of which 35.7 per cent were graded correctly. The percentage correctly graded was 42.7 for 159 samples in all four storage groups. Other grades erroneously called "fair" consisted of "excellent" 0.7 per cent, "good" 16.3 per cent and "poor" 22.6 per cent, giving a total of 12 per cent from 365 samples. These results seem to indicate that butter from cream scoring 90 to 91 cannot be accurately graded by the microscopic method. Factors such as chemical action and absorbed flavors apparently have a more important rôle in lowering cream to "fair" grade than was the case in the two higher grades of cream.

TABLE 3

*Butter churned from "fair" cream scoring 90 to 91*

	Storage Period			
	1-3 days	7 days	14 days	30 days
Total Samples .....	57	20	40	42
Number graded right .....	25	12	16	15
Number graded wrong .....	32	8	24	27
Number missed 1 grade too high .....	19	3	9	13
Number missed 2 grades too high .....	2	5	15	1
Number missed 1 grade too low .....	11	0	0	13
Per cent graded right .....	43.9	60.0	40.0	35.7

Table 4 shows the results for cream scoring below 90, "poor" grade. Seventeen samples were held 1 to 3 days and 47.0 per cent graded properly. Of the 10 held 7 days 70 per cent were given the correct grade. For 14 days 62 samples were stored, of which 54.8 were graded accurately. A percentage of 38.5 was correctly graded for the 8 samples stored 30 days.

A summary of the four groups shows that 97 samples were examined and

TABLE 4

*Butter churned from "poor" cream scoring below 90*

	Storage Period			
	1-3 days	7 days	14 days	30 days
Total Samples .....	17	10	62	8
Number graded right .....	8	7	34	3
Number graded wrong .....	9	3	28	5
Number graded 1 grade too high .....	8	1	11	2
Number graded 2 grades too high .....	1	1	15	2
Number graded 3 grades too high .....	0	1	2	1
Per cent graded right .....	47.0	70.0	54.8	38.5

53.6 per cent of these correctly graded. The "excellent" grade erroneously ascertained as "poor" was 3.4 per cent, "good" 15.1 per cent and "fair" 27.6 per cent, making a total of 15.9 per cent for 427 samples. The microscopic examination was successful in determining the grade for "poor" cream in only about fifty per cent of the cases. This was explained by the fact that many times a large number of the micro-organisms had completely decomposed, thus giving the appearance of a higher grade.

Altogether 524 samples composed the entire study of the four grades. The percentage determined correctly equaled 61.8.

A comparison was conducted on the same butter, one sample held 1 to 3 days and the other 30 days. A total of 103 churnings was studied consisting of "excellent" "good" and "fair" grades. In both storage periods the same percentage of 62.2 was graded correctly, indicating neither holding period was advantageous. A change occurred in the butter micro-flora during the 30 day holding, but this change caused some samples to be graded correctly that were missed in the 1 to 3 day storage and vice versa.

Culture organisms were added to approximately half the entire samples studied. The conclusions drawn from the results on the four grades indicated that the presence or absence of culture organisms did not influence the accuracy of the microscopic determinations. The number of culture organisms was considerably greater in butter from "excellent" cream than from "fair" cream when adding the same amount. This seemed to verify the observation that culture has more effect on high quality cream than on poorer cream.

Samples of the "fair" grade were obtained from commercial plant operations to compare with those churned by the laboratory procedure. Of the 55 commercial samples 30.9 per cent were correctly graded and 48.1 per cent of the 106 laboratory samples. The difference was not considered large enough to more than suggest that the commercial samples might be a trifle harder to grade correctly.

A study of the data on butter slides for a possible correlation between certain micro-organisms and specific flavor defect in cream was negative. The only means of distinguishing among the stained micro-organisms was in size, shape, and whether they occurred singly, in pairs, or in clumps. This was insufficient information to correlate organisms with specific flavor defect in the cream.

#### SUMMARY

The data recorded in the study consisted of sample number, cream score, criticisms of flavor, appearance of the cream slide, and appearance of the stained butter serum.

In the preliminary work 130 samples were studied for the purpose of determining the microscopic appearance of the butter serum. This informa-

tion was correlated with each of the four grades of cream from which the butter was churned. The grades of cream used in making the butter were "excellent" scoring 93 or above, "good" 91½ to 92½, "fair" 90 to 91, and "poor" below 90 in score.

The stained slides of 524 samples of butter were studied under the microscope and the grade of cream predicted. Of these 524 samples, 143 were from the "excellent" group and 131 (91.6 per cent) were given the correct grade. In the "good" group there were 125 samples. Seventy-three (58.4 per cent) were accurately graded. One hundred and fifty-nine samples of the "fair" group were examined and 68 (42.7 per cent) had the right grade predicted. In the last group 52 (53.6 per cent) of the 97 "poor" group samples were graded accurately. This seems to indicate that the microscopic examination was fairly accurate in distinguishing butter made from "excellent" cream, but for lower grades it was not a reliable method.

Storage periods of 1 to 3 days, 7 days, 14 days, and 30 days did not materially influence the number of grades determined correctly. This was verified by a trial in which one-half the samples was held for 1 to 3 days and the remaining samples 30 days. The percentage determined correctly from 103 churnings was the same (62.2) for both storage periods.

The presence or absence of culture organisms did not effect the microscopic grading.

The results on studies of the "fair" group indicated samples from commercial churnings were slightly more difficult to grade correctly than those from the laboratory procedure. This was apparently caused by the difference in handling conditions.

The two apparent reasons for not being able to determine the "good," "fair," and "poor" grades as accurately as the "excellent" grade were: (1) Low scoring cream was not always the result of bacteriological action but was caused by chemical action and absorbed feed flavors. (2) The other reason was the contamination by organisms subsequent to pasteurization of cream and during the churning process.

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## NOTE ON VIOLET RED BILE AGAR FOR DETECTION OF *ESCHERICHIA COLI*

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Since most of the reported work using Violet Red Bile Agar for the detection of *Escherichia coli* has been on a theoretical laboratory scale, herewith is reported a practical application that yielded such satisfactory results that it appears worthy of mention.

A dairy, located in one of the North Central States, was having difficulty with a re-contamination by *Escherichia coli* after the milk received a heat treatment of 170° F. for 30 minutes. The finished product of this dairy could not be marketed unless the material was free from *Escherichia coli*. It was feared that perhaps the organism was getting into the heated milk from the spray pond water which was used as condensing water in the evaporator and dryer. In an attempt to trace this source of *Escherichia coli*, the authors were invited as consultants.

Samples of the milk, secured at various points in the process after the milk had been heated as described above, were tested for the presence of *Escherichia coli*. Two different methods were used for the detection of the organism; namely, (1) inoculating the milk into Brilliant Green Bile Broth 2% Medium for the presumptive test followed by streaking on Eosin-Methylene Blue Agar from the tubes that showed gas production, and, (2) plating the milk directly, using Violet Red Bile Agar as the medium. On the last named medium the organism produces small, purplish red colonies which are surrounded by a zone of precipitated bile after an incubation period of 18 to 24 hours at 37° C.

Over a hundred samples were tested for the presence of *Escherichia coli* and in every instance when typical colonies of this organism developed on the Violet Red Bile Agar in 18 to 24 hours, corresponding, confirmed-positive results were obtained in 48 hours using the other method. On the other hand, when gas was produced in the Brilliant Green Bile Broth which gave a spurious test on the confirmation medium, no typical colonies were produced on the Violet Red Bile Agar, so that, during the practical application of these two methods in the detection of *Escherichia coli*, perfect correlation was obtained.

Using the direct plating method that gave results in 18 to 24 hours as compared to the 48 hours required by the Brilliant Green Bile Broth and Eosin Methylene Blue Agar method, changes could be made within 24 hours after the samples were taken to correct or eliminate conditions that were a

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possible source of re-contamination. Whereas if the results were not obtained before 48 hours, no changes could have been started until two days had elapsed after the samples were taken. Hence much time and expense were saved by accepting the results of the Violet Red Bile plates which enabled the necessary corrections and alterations to be made before the day's manufacturing started thereby maintaining the daily production schedule.

The results obtained raise the question, "When *Escherichia coli* did make its appearance was the recontamination sufficiently heavy to make both of these methods equally sensitive?" In this study no attempt was made to determine the relative sensitivities of these two media. Nevertheless, the fact remains that, using the results obtained with Violet Red Bile Agar to solve the practical problem described, much time and material were saved.

# LEUCOCYTES AND THE METHYLENE BLUE REDUCTION TEST<sup>1</sup>

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The observation is not infrequently made that some samples of milk apparently of low bacterial content have unexpectedly short reduction times. It has been customary to explain this phenomenon by assuming a reducing activity for milk leucocytes, although unequivocal acceptance of this explanation is not always possible in the absence of any more adequate measure of bacterial numbers than the methylene blue reduction test itself. In the present study an attempt has been made to use more delicate criteria of the influence of bacteria on the reduction times of such milks than the plate count or a cursory preliminary microscopic examination of the milk.

## HISTORICAL

Skar (6) demonstrated reduction of methylene blue in sterile milk by leucocytes from the lymph gland of a steer. He believed that a leucocyte content up to approximately 6.7 million per cc. cannot reduce methylene blue in milk in the standard test but that leucocytes effect reduction of the dye if they are kept evenly distributed by periodic agitation of the milk during incubation.

Barthel (1), although recognizing the reducing power of leucocytes, is not inclined to attribute to this power great importance in milk control.

Wilson (9) concurs in the opinion that leucocytes are a factor in the reduction test but was unable to effect any marked decrease in "aerobic" reduction times of milks to which suspensions of rabbit leucocytes were added.

Ramsdell (5) observed a general but not direct relation between the reduction of resazurin and the leucocyte content of milk but was unable to demonstrate reduction of either resazurin or methylene blue by washed leucocytes. He believed "the cause of reduction must be the result of the presence of substances associated with cells, or substances present in abnormal milks in amount comparable to the cell content."

Devereux and Bryan (2) and Hastings (4) regard leucocytes as being significant in the reduction test, at least in better class milks.

## METHODS

The technique of collection and analysis of the milk samples is described elsewhere (7) and will not be repeated here. Plate counts are not reported

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<sup>1</sup> The data contained herein are taken from a thesis presented by N. J. Strynadka (now Inspector of Dairy Products, Dairy and Cold Storage Branch, Dominion Department of Agriculture) at the University of Alberta in partial fulfilment of the requirements for the degree of Master of Science.

as they were found to contribute practically nothing to the study. The Breed counts refer to bacteria only and the number of examined fields from which they were computed is in each case cited except when the bacterial content was in the millions in which case usually 5 microscopic fields per Breed smear were counted.

The leucocyte counts are computed from the examination of 60 microscopic fields per Breed smear except when the count was very high in which case fewer fields were observed. All cells other than the cells of microorganisms were classed as leucocytes and differentiation of kinds of leucocytes was not attempted. All bacterial and leucocyte counts are on a per cc. basis and the methylene blue reduction times are reported in hours and minutes, 1:45 meaning 1 hour and 45 minutes.

#### CORRELATION BETWEEN LEUCOCYTE COUNTS AND REDUCTION TIMES

The coefficient of correlation between the leucocyte counts and the standard methylene blue reduction times of 158 samples of aseptically-drawn milk was found to be  $0.698 \pm 0.027$ . This constant as a sole criterion is not sufficiently high, in the opinion of the authors, to justify the popular assumption that milk leucocytes reduce methylene blue in milk.

#### THE INITIAL BREED COUNT

The initial 1000 field Breed counts are available for 95 of the 158 samples of milk and show that in the large majority sufficient bacteria were probably present to account for reduction through bacterial action irrespective of the leucocyte count which sometimes was very high. There were a few exceptional samples having leucocyte counts over 1 million, reduction times of less than 10 hours and low Breed counts. There were also 6 samples with low leucocyte and Breed counts and short reduction times. It is probable that the initial 1000 field Breed count when applied to this type of milk is not a sufficiently precise measure (7) to justify a conclusion from the foregoing data further than that in the majority of the samples the bacterial content was high enough to account for reduction of the dye independently of the leucocytes.

#### THE BACTERIAL CONTENT AT THE TIME OF REDUCTION

It has been shown that in samples of this class of milk about which there is no suspicion of abnormality the bacterial content at the moment of reduction is many millions per cc. as measured by the Breed count (8). It would appear sound to assume that in such samples the only practically significant reducing influence is exerted by the bacterial cells. In our present state of knowledge the number of bacteria at the time of reduction seems to be the most precise criterion of the preponderating influence of the bacteria on the reduction time.

The Breed counts at the moment of reduction were found to be over 60 million in 34 of 46 samples of aseptically-drawn milk. The reduction times of 19 of these samples were less than 10 hours but the Breed counts on reduction of 7 of these samples were over 60 million. The remaining 12 milks (tables 1 and 2) are interesting exceptions.

TABLE 1

*Ten milks reacting abnormally to the reduction test*

Milk number	Reduction time	Leucocyte count	Initial 2000-field Breed count	2000-field Breed count at reduction	60-field Breed count after total of 8 hours incubation
1	0:30	47,400,000	477,000	68,400	590,000
2	0:45	17,800,000	107,000*	253,800*	
3	0:55	8,300,000	142,000	86,000	670,800,000
4	1:45	4,160,000	119,700	364,200	
5	1:45	5,340,000	91,200	59,700	
6	1:45	15,960,000	48,000	43,000	6,000,000
7	2:10	7,520,000	335,000	146,000	920,000
8	3:30	4,150,000	200,700	56,400	140,000
9	5:00	680,000	39,200	81,600	210,000
10	7:15	3,500,000	56,100	435,600*	

\* 1000-field count.

The initial plate counts of these 12 exceptional milks varied from 50 to 7600 per cc. but the initial Breed counts were in some cases rather high. The 2000 field Breed count at reduction was in only 1 sample over 0.5 million while the leucocyte counts were low (less than 1 million) in 2 samples.

These data are not clear-cut evidence that the leucocytes possessed a reducing power. From the point of view of numbers only it is difficult to attribute to 4 million leucocytes in one milk a reducing power of 1:45 and to 3.5 million in another a reducing power of 7:15 while it took 16 million in a third 1:45 to reduce the dye. Samples 9 and 12 were low in leucocytes and in bacterial content at reduction. When these two milks are considered with the six exceptional milks mentioned in the preceding section entitled *The Initial Breed Count* (one of which had a reduction time of 6:45, an initial 1000 field Breed count of 6000 and a leucocyte count of 180,000) the reducing power of the leucocytes is not a tempting explanation of the short reduction times.

For research purposes in this laboratory the standard methylene blue reduction test is routinely supplemented by the modified reduction test (hourly or half-hourly agitation of the tubes during incubation). Occasionally samples of aseptically-drawn milk were encountered which had considerably longer modified than standard reduction times. In each case where the particulars of the animal giving such milk was available a history

TABLE 2  
*Two milks reacting abnormally to the reduction test*

Milk No.	Reduction time		Leucocyte count	Initial Breed count	Fields examined	Breed count at reduction	Fields examined	60-field Breed count after 8 hrs. incubation	Breed count at (modified) reduction
	Stand-ard	Modi-fied							
11	5: 00	7: 15	2,100,000	143,100	2,000	1,017,600	1,000	18,660,000	127,200,000
12	6: 15	14: 45	860,000	75,000	1,000	132,800	2,000	350,000	64,200,000

of udder abnormality was found. In two such milks, reported in table 2, it is evident that bacterial action was responsible for the modified but not the standard reduction times.

These results do not confirm Skar's theory that shortened reduction times in the modified test are due to the more even distribution of the leucocytes because of agitation. A few samples of aseptically-drawn milk of short standard reduction times, including samples 11 and 12, were encountered which when shaken immediately after reduction had second reduction times varying up to 10 to 12 hours. It is, indeed, difficult to accept the reducing power of the leucocytes as the explanation of these observations.

It is doubtful if the 2000 field Breed count is sufficiently accurate to justify the conclusion that phagocytosis was responsible for lower Breed counts at the time of reduction than initially in any of these milks. It is possible that bacterial reproduction was continuous in all of these milks but was not apparent because of phagocytic action of the leucocytes. Nothing was observed that caused suspicion that this phenomenon was operative and the modified reduction times of milks 11 and 12 are not in support of such a theory.

#### THE ADDITION OF LEUCOCYTES TO MILK

In 1913 Skar (6) reported the reduction of methylene blue in sterile milk by an added suspension of leucocytes from the lymph gland of a steer. Gay

TABLE 3  
*The reduction times of milk plus bovine blood leucocytes*

Tube number	Description of samples	Modified reduction time	Breed count at reduction
1	Milk only	12:30	
2	" "	12:45	
3	" "	13:15	
4	9 cc. milk + 1 cc. leucocyte suspension*	11:00	200,000,000
5	9 cc. milk + 1 cc. from Tube 4	11:45	
6	9 cc. milk + 1 cc. from Tube 5	12:15	
7	9 cc. milk + 1 cc. leucocyte suspension*	10:45	190,000,000
8	9 cc. milk + 1 cc. from Tube 7	11:30	
9	9 cc. milk + 1 cc. from Tube 8	12:45	
10	9 cc. milk + 1 cc. blood serum	12:00	120,000,000
11	9 cc. milk + 1 cc. from Tube 10	12:00	
12	9 cc. milk + 1 cc. from Tube 11	12:45	
13	9 cc. milk + 1 cc. red blood cell suspension	11:00	126,000,000
14	9 cc. milk + 1 cc. from Tube 13	12:30	
15	9 cc. milk + 1 cc. from Tube 14	12:30	

\* By computation this milk leucocyte suspension mixture contained approximately 50 million leucocytes per cc.

and Oram (3) demonstrated the reduction of methylene blue in sterile broth in the presence of leucocytes. The failure of the leucocytes to reduce methylene blue similarly in the presence of a streptococcus filtrate was ascribed to a leucocyte-destroying activity of "streptococcus leucocidin."

In the present study blood was drawn aseptically from the jugular vein of a Jersey cow into a flask of sterile physiological saline solution. The leucocytes were separated by repeated centrifugalization, decantation and washing in sterile saline solution until finally a clear suspension of approximately 500 million leucocytes per cc. was obtained. This suspension as well as clear serum and a suspension of the separated red blood cells was added to milk drawn aseptically from the udder of the same cow in varying proportions as outlined in table 3. The animal from which the blood and milk were drawn had not had a recognized udder abnormality while the milk had a standard reduction time of approximately 25 hours and a leucocyte count of 330,000. Only modified reduction times are reported in tables 3 and 4 because the normal variation of reduction times in replicate tubes of this milk militates against an intelligent interpretation of the standard reduction time results. It cannot be said with certainty that either the added leucocytes, red blood cells or blood serum had a measurable effect on reduction times. Whatever effect there may have been was small and was the same for red blood cells as for leucocytes. Sufficient bacteria were present at reduction to account for reduction.

Efforts to stain the leucocytes after their addition to the milk failed. It was thought that the loss of staining properties might be accompanied by loss of reducing power. In an endeavor to study the effect of adding to milk leucocytes retaining their staining properties 200 cc. of sterile physiological saline solution were injected intraperitoneally into a rabbit early in

TABLE 4  
*The reduction times of milk plus rabbit leucocytes*

Description of samples	Modified reduction time		Leucocyte count	Breed count at reduction
	Tube			
	1	2		
45 cc. milk + 10 cc. leucocyte suspension	11: 00	12: 00	1,120,000*	66,400,000
45 cc. milk + 7 cc. leucocyte suspension	11: 00	12: 00	730,000	68,400,000
45 cc. milk + 3 cc. leucocyte suspension	11: 00	11: 00	590,000	77,200,000
45 cc. milk + 0.5 cc. leucocyte suspension	10: 30	10: 30	520,000	70,400,000
Milk only	10: 00	10: 30	340,000	
45 cc. milk + 10 cc. saline solution	11: 00	11: 00		84,000,000
45 cc. milk + 5 cc. saline solution	10: 30	11: 00		

\* By computation this milk leucocyte suspension mixture contained 2,096,363 leucocytes per cc.

the morning followed by a further injection of 100 cc. late in the afternoon. Three hours later 200 cc. of exudate were removed by aspiration. By centrifugalization, decantation and washing in sterile saline solution a suspension containing approximately 10 million leucocytes per cc. was finally obtained. Milk from the same animal as previously used was treated immediately as outlined in table 4. It is seen that the leucocytes retained their staining properties in fair degree after their introduction into the milk but no decrease in reduction time is noticeable.

Since the completion of these experiments Wilson (9) reported reduction times of raw and pasteurized milks to which varying concentrations of rabbit leucocyte suspensions were added. Although he believes that reduction by leucocytes was demonstrated, he observes that "the results were a little irregular" and "extremely difficult to understand."

#### DISCUSSION AND CONCLUSIONS

The mere presence of leucocytes in milk, even in large numbers, and the absence of bacteria in large numbers do not prove a reducing power for the leucocytes. The attempts to furnish proof of such a reducing power have to date depended on strictly quantitative measurements and in the opinion of the writers have been unsuccessful. The need for qualitative measurements seems apparent.

The observations reported in the literature and in this paper are explicable in terms consistent with accepted theories of dye reduction in milk. There are reasons for believing that the abnormal udder conditions responsible for milk of high leucocyte content are also responsible for abnormally high concentrations of reducing substances in the milk. The presence of reducing substances in abnormally high concentrations would explain the observations under discussion without soliciting aid from the leucocytes. This is not, of course, a denial of the possibility of some leucocytes possessing reducing properties but the bulk of the evidence is that leucocytes are rarely, if ever, the main or significant influence in the reduction of methylene blue in milk in practice.

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# THE VISCOSITY OF ICE CREAM MIX MADE WITH PLAIN AND SUPERHEATED CONDENSED SKIM MILK

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Ice cream manufacturers at one time were convinced that good ice cream mix should be viscous. Processing mix in homogenizers was not a common practice at that time and it is not unlikely that a viscous mix was desirable under the freezing, storage, and handling conditions which prevailed. With the wider use of the homogenizer, better freezers and better methods of distribution, it was later demonstrated that high viscosity was of no special value and in recent years the trend has been towards mixes of low viscosity.

Various methods of increasing the viscosity of mix were employed at the time viscous mixes were deemed desirable, among them being the use of superheated condensed products. Superheated condensed milk is not widely used at the present time, probably because of its influence on the viscosity of the mix, difficulties in handling it, and its slight "cooked" flavor. However, some manufacturers find its use desirable, as it produces a type of ice cream which is popular in some localities.

Relatively little has been written on the use of superheated condensed milk in ice cream. Tracy (1) found an improvement in texture and body of the ice cream and its resistance to melting when superheated solids were used. He also noted an increase in the mix viscosity. Williams and Hall (2), using a sales-preference test as a means of determining desirable types of ice cream, found that the use of superheated condensed skim milk produced a better ice cream than plain condensed. Johnson and Ward (3) have shown that the viscosity of superheated condensed milk is not a true index of the value of milk for bread making, but observed that it is superior to condensed milk which has not been superheated. They state that it is the heat treatment accorded the milk which operates to improve the baking quality of the milk, and this may or may not be reflected in the viscosity of the product.

In the manufacture of superheated condensed milk, the temperature is raised to 185-195° F., by injecting live steam into the product before removing it from the vacuum pan. This treatment causes a thickening of the product, the degree of thickening depending on the time and temperature of heating. The heating is discontinued at the point of maximum thickening as any overheating results in a coarse coagulation of the product. Over cautious and inexperienced operators usually stop heat-

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ing before the point of maximum thickening, and thus the benefits of properly superheated milk are only partially obtained. Since superheating, when properly conducted, brings about a marked increase in viscosity, it is to be expected that the beneficial effects of superheating are often attributed to the increase of viscosity and this property is frequently used as a measure of its value for ice cream.

The viscosity of ice cream mix and its influence on the freezing properties and quality of the finished ice cream has been studied by a number of investigators. Leighton and Williams (4) first showed that ice cream mix had an "apparent" viscosity after proper aging and that agitation, such as is given the mix in the freezer, reduced this to a lower value which they termed "basic" viscosity. Following the work of these authors, most investigators used the term "basic" viscosity as that which results when the mix is strongly agitated as in the freezer or with special devices designed for this purpose (6), although Hening (5) recommends homogenization of aged mix as a means of obtaining the "basic" viscosity. It is probable that the action of the homogenizer is more drastic than the dashers of a freezer and, therefore, the method of Hening may give viscosity values below those which are of actual interest in the manufacture of ice cream.

During the course of some investigational work on superheated condensed skim milk, it was observed that the viscosity could be markedly reduced by homogenization. The terms "apparent" and "basic" viscosity, suggested by Leighton and Williams (4) for ice cream, were used to describe the viscosity of this product before and after homogenization.

It is the purpose of this paper to record the observations made during a study of the effect on ice cream of using superheated condensed skim milk which had been reduced by homogenization to its basic viscosity in comparison with plain condensed skim milk and with superheated which had not been treated to reduce its viscosity.

#### EXPERIMENTAL

*Determination of viscosity:* In this work the viscosity of the various products was determined by means of a Saybolt viscosimeter having an orifice of 0.082" diameter. The time required for 60 cc. of the product to run through at the designated temperature was taken as the viscosity. The Saybolt tube was mounted in a water bath to insure accurate temperature control. All samples were aged over night at 40°F. before viscosity determinations were made.

*Preparation of the condensed products:* Plain condensed skim milk and superheated condensed skim produced in a commercial plant were used throughout this experimental work. In preparing the homogenized superheated product, the material was passed through a single stage homogenizer

at a temperature of 125° and at pressure of 3000 lbs. per sq. in. and held a day at 40° before being used in the mixes.

*Preparation of the mixes:* All of the mixes made in this experiment were of the following composition:

Butterfat	13%
Milk solids not fat	11%
Sugar	15%

All of the milk solids not fat not supplied by the whole milk (4% fat) and cream (40% fat) were obtained from the condensed product under consideration. In those mixes made with gelatin, a gelatin of 160 Bloom was used in the amount of 0.4%. The mixes were compounded in the usual manner, pasteurized at 150° F. for 30 minutes, and homogenized in a two stage homogenizer using 2000 lbs. on the first valve and 1000 lbs. per sq. in. on the second valve, which gave a total of 3000 lbs. per sq. in. The mixes were cooled to 40°F. and aged over night.

Since the primary purpose of this study was to observe the influence of the viscosity of the condensed product on the viscosity of the mix, not all of the mixes were frozen into ice cream. However, a few of the mixes were frozen and for this a 40 qt. batch freezer was employed, the ice cream being drawn at 90% overrun. A record was made of the time required to reach this overrun and also of the general quality of the finished product after three days in the hardening room.

#### RESULTS AND DISCUSSIONS

*Viscosity of the condensed products:* Typical data on the viscosity of the condensed products and the resulting mixes are summarized in Table 1. It will be seen that when superheated condensed skim was homogenized a marked reduction in viscosity was obtained. Under the conditions of this experiment the viscosity of the homogenized superheated product was greater than that of the plain condensed but the difference, although noticeable, was small in comparison with the difference between the plain and the unhomogenized superheated. The superheated condensed which had been homogenized was of such viscosity that it could be handled easily, pouring readily and draining rapidly from cans.

*Viscosity of mixes made without gelatin:* Inspection of the data in Table 1 indicates that in those mixes made without gelatin there was practically no noticeable difference between the viscosity of the three mixes. The high apparent viscosity of the superheated condensed does not carry over into the mix, showing that the homogenization process given the mix is effective in reducing the viscosity of the mix to its basic viscosity. The small difference noted in both the apparent and basic viscosity between the one mix containing plain condensed skim milk and

the two mixes, made with superheated condensed, was due to the heat treatment these two products received, but since there was practically no difference between the mixes containing the homogenized and unhomogenized condensed, it may be said that the beneficial effects of superheating cannot be accurately measured by its viscosity.

TABLE 1

*The viscosities of plain, superheated and homogenized superheated condensed skim milk and ice cream mixes made with these products*

Kind of product	Total solids of product %	Viscosity of product at 50° F. Secs.	Viscosity at 40° of ice cream mix made with each source of M.S.N.F.			
			No gelatin used in mix		0.4% gelatin used in mix	
			Apparent viscosity Secs.	Basic viscosity* Secs.	Apparent viscosity Secs.	Basic viscosity* Secs.
Plain condensed skim milk	28.7	54	54	52	508	201
Superheated condensed skim milk .....	28.4	more than 36,000	69	67	about 1000	331
Homogenized superheated condensed skim milk .....	28.4	446	68	67	about 1000	336

\* Mixes reduced to basic viscosity by means of a laboratory agitator in absence of air (6).

*Viscosity of mixes made with gelatin:* An amount of gelatin (0.4%) was used in these mixes to make a definite gel. From the data presented in Table 1 it will be seen that the presence of the gelatin increased the viscosity of the mix. The influence was chiefly on the apparent viscosity, which was comparatively high. However, the gelatin also increased the basic viscosity to a noticeable degree. The same essential differences in apparent and basic viscosity existed between the three mixes as was found in the mixes made without gelatin.

*Freezing properties of the mix and quality of the ice cream:* Very little difference in freezing time was noted in the few mixes which were frozen and the differences were not considered of commercial importance. It may be said, however, that in every case mixes made with plain condensed skim milk whipped very slightly faster than the other two mixes in the series.

In comparing the general quality of the three ice creams made in this series, it may be said that the ice cream made with the plain condensed skim was somewhat less smooth in texture and slightly weaker in body

than the two ice creams containing the superheated condensed products. This sample also differed from the other two in that it had a slightly less cooked flavor. As there was no difference in the texture, body and flavor between the ice cream made with the superheated condensed and the homogenized superheated condensed, it is evident that the homogenization of the condensed product had no effect on its value for ice cream as far as quality of the ice cream was concerned.

#### SUMMARY

Mix made with superheated condensed skim milk is but slightly more viscous than mix made with plain condensed skim milk.

The high apparent viscosity of superheated condensed skim milk can be reduced by homogenization to such an extent that the product may be easily poured and handled.

Reduction of the viscosity of superheated condensed by homogenization in no way influences the viscosity of ice cream mix.

The viscosity of superheated condensed is not a true measure of its value in ice cream mix.

Whatever beneficial effects are obtained by using superheated condensed skim milk, they are not the result of the higher viscosity, but are probably due to the high heat treatment accorded the product.

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# THERMAL SHOCK RESISTANCE OF MILK BOTTLES

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The presence of significant numbers of thermal shock cracked bottles in the daily rejects of two large dairies was reported in a previous study (1). The present study was made in order to better understand the relation of thermal shock resistance of bottles determined by test to their failure in actual usage.

The thermal shock test for milk bottles was first introduced into the dairy industry in this country by Kouwenhoven (2) in 1926. The routine usage of a thermal shock test in the Chicago milk bottle exchange was reported in 1934 (3). Private concerns have used some form of the test for a number of years. The origin of the test in the glass business is probably lost in the antiquity of the art. Some effort has been made in recent years to study the limitations of the test and to standardize a method. It was announced in the spring of 1937 that the Glass Container Association had authorized a research project to develop standard bottle tests including the thermal shock test.

Although there appears to be some disagreement among glass technologists as to whether or not the thermal shock test is a general test for the strength of bottles, it is well agreed that much can be learned through its use about their thermal endurance in commercial usage. Inasmuch as the resistance of glass to compression is much greater than its resistance to tension, it is believed that tensile stresses are chiefly responsible for thermal, as well as impact breakage (4). The thermal shock test made by rapidly cooling hot bottles over various temperature ranges, applies a controlled trial tension to the bottle.

The thermal shock test used in this study was developed after much preliminary experimentation and was found to give reproducible results on representative samples of commercial bottles. The test is naturally somewhat empirical and is subject to human errors. All the tests reported here were made by one person who exercised all possible care.

## THE THERMAL SHOCK TEST

Thermal shock tests were made in two square galvanized iron sinks approximately 36 inches long, 24 inches wide, and 24 inches deep. One was heated directly by gas flame and the other was kept cold by a small stream of cold water. The hot tank was equipped with a mechanical agitator and the cold tank was equipped with a constant level drain. The temperatures

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were controlled to within 1°F. of the desired temperature. The hot tank had a false bottom of wood slats to prevent the bottles from resting on the heated metal surface. The depth of the water in each tank was regulated so that the bottles in an upright position were covered by 1½ to 2 inches of water. The bottles to be tested were placed individually, using tongs, in the hot tank, allowed to fill slowly to warm, then emptied and submerged again. All the bottles were thus placed in the hot water at intervals of one minute. After 10 minutes or longer had elapsed, starting with the first and continuing in order at one minute intervals, the bottles were lifted out, inverted to empty, and plunged vertically bottom downward to the bottom of the cold water tank as rapidly as possible allowing them to fill under water. This operation required less than 5 seconds. As soon as all the bottles had been shocked they were carefully examined individually for cracks. Many of the cracks found were very fine and not very deep in the surface of the glass. Direct sunlight was more satisfactory than diffuse sunlight or incandescent-lamp light for the examinations. It was found that if the shocked bottles remained too long in the cold water or air after shocking the fine cracks closed and became invisible. The examinations were made, therefore, as soon as possible after the shocking.

### *The effect of thermal differential on the resistance of quart bottles to thermal shock*

Hot tank °F.	Cold tank °F.	Diff. °F.	No. of bottles tested	Cracked		Location of cracks
				No.	%	
<i>Lot A</i>						
124	44	80	102	1	1.0	1—Lip
129	44	85	132	2	1.5	2—Lip and neck
134	44	90	40	3	7.5	3—Lip and neck
138	43	95	30	7	23.0	5—Lip and neck 1—Shoulder 1—Bottom
145	45	100	20	6	30.0	4—Lip and neck 2—Bottom rim
147	42	105	19	9	47.0	6—Lip, neck, sides & bottom 2—Lip and neck 1—Bottom
<i>Lot B</i>						
126	66	60	47	1	2.1	1—Lip
120	50	70	25	3	12.0	3—Lip and neck
130	50	80	20	4	20.0	4—Lip and neck
140	50	90	15	8	53.0	6—Lip and neck 2—Bottom rim and sides

## I. RELATION OF TEMPERATURE DIFFERENCE TO CRACKING

Two gross of new quart bottles of one make (Lot A) were carefully examined individually and the few damaged and flawed bottles found were discarded. Previous work had shown that the presence of surface defects reduces the normal resistance to shock. After thoroughly mixing the lot of bottles, sample portions were tested at thermal differentials varying from 80°F. to 105°F. in steps of 5°F. The results are shown in Table 1. The relation between thermal differential and percent of bottles cracked in this experiment is shown graphically in Fig. 1.

Another lot of bottles of the same make, but manufactured at a later date (Lot B), were tested in a similar manner. They were sampled from 7 gross of new bottles used in the bottle washer thermal shock experiments which will be described in Part III. Since they were found to be consider-

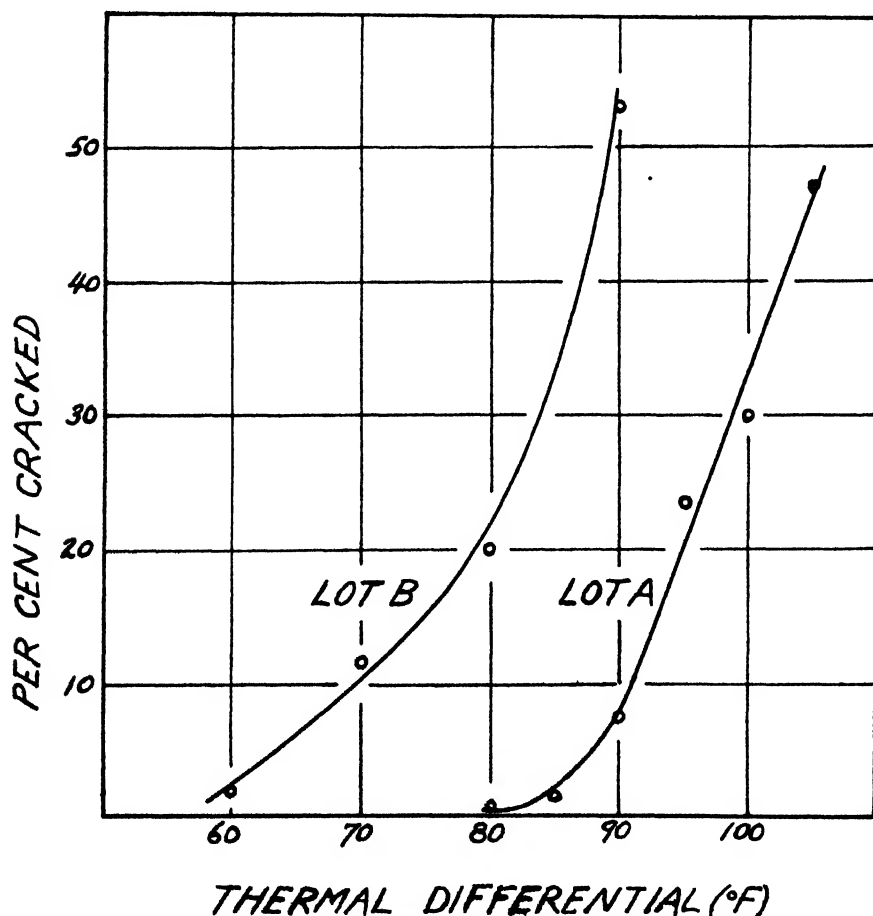


FIG. 1. Relation between thermal differential and per cent of cracked quart bottles in the thermal shock test.

ably less resistant to thermal shock than the first lot tested, the thermal differentials used were from 60°F. to 90°F. in steps of 10°F. The results are shown in Table 1. The relation between thermal differential and percent of bottles cracked is shown graphically in Fig. 1.

In each curve there is a leveling off below 10% cracked, indicating that there is a small percentage of bottles very low in thermal shock resistance. It should be pointed out that a range of bottle breakage as low as 0.1% to 1.0% is of economic importance in commercial milk plant operation. The cracks in these bottles were in the outer surface of the glass. In general, the cracks at low thermal differentials were shorter and more difficult to see than those at the higher differentials. These short fine cracks have been observed in newly washed bottles in commercial operations by individual inspection. The inspectors at the washer discharge almost invariably fail to see them. It will be noted that cracks did not develop in the bottom or side regions of the bottles except at greatest thermal differentials. This is contrary to the results obtained by Murgatroyd (5) who found that bottles cracked in the thermal shock test only in the wall near the base (bottom rim). In the test he used, the cold bottles standing in cold water up to the lip were filled with hot water. It is questionable whether the lip region is shocked as severely, by this method, as is the lower region. Considering the average cracking temperature or the temperature at which 50% were cracked, Lot A had a 15°F. favorable margin over Lot B.

## II. EFFECT OF REPEATED THERMAL SHOCKS

A representative sample, consisting of 30 bottles, of a shipment of new quarts were tested at a differential of 90°F. (140° to 50°). The whole test was repeated a total of 20 times examining the bottles between each test and noting the effects of each shock. The results are recorded in Table 2.

The twenty bottles from Lot B, tested at 80°F. differential in Part I, were retested at 80°F. differential for 9 more consecutive times with examination between as previously described. The results are shown in Table 3.

It appears that repeated shocking resulted in continued breakage up to a limit which differed for the two lots tested. Murgatroyd (5) reported a similar effect of repeated shocking and attributed it to the inaccuracy of the test. It would appear that this effect is related to the inherent nature or weakness of the bottles and may be a type of fatigue influenced by the time element or the number of shocks. The cracks in most of the bottles were associated with the tiny impact marks which result when bottles strike together. There were impact marks, however, on the bottles which survived the shocking. This seems to indicate that the impact marks help determine the location or path of the cracks. Cracks in the bottoms and bottom rims of the bottles did not appear until the bottles were shocked a number of times. This region was also more resistant in the temperature differential tests.

TABLE 2  
Thermal shock cracking of new quart bottles (Lot A) during 20 successive shocks at 90° F. differential

Bottle No.	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	17th	18th	19th	20th
1	0	0	0	0	0	0	0	0	0	0	0	0	R, X	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	R, X, D	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	X	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Cracked-Cumulative	1	3	4	6	6	8	8	10	14	16	16	18	20	22	23	24	25	25	25	25

Key to symbols:

L = Crack in lip.

N = Crack in neck.

S = Crack in side.

R = Crack in bottom rim.

B = Crack in bottom.

0 = No cracks.

X = Crack extended.

- = No change.

D = Discarded bottle.

TABLE 3

*Thermal shock cracking of new quart bottles (Lot B) during ten successive shocks at 80° F. differential*

Bottle No.	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	L, N	X	D							
6	L	L, X, N	-	-	-	X	-	R, D		
7	0	0	0	0	0	R	-	-	-	-
8	0	L	N	X	-	X	X	-	-	-
9	L, N	-	X	R, D						
10	0	0	L	-	-	-	-	-	R, D	
11	0	0	0	0	L	-	-	-	-	-
12	0	0	0	0	0	0	0	0	0	0
13	0	L	X	X	-	-	-	-	S, D	
14	0	L, N	X	R	D					
15	0	L, S	D							
16	0	0	0	0	0	0	L	-	-	-
17	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	L	X	X	-	-	-
19	0	0	0	0	0	0	0	0	0	0
20	L	-	-	-	-	-	-	-	-	-
Total Cracked-Cumulative	4	8	9	9	11	12	13	13	13	13

**Key to symbols:**

L = Crack in lip.

N = Crack in neck.

S = Crack in side.

R = Crack in bottom rim.

B = Crack in bottom.

0 = No cracks.

X = Crack extended.

- = No change.

D = Discarded bottle.

### III. THERMAL SHOCK CRACKING OF BOTTLES DURING WASHING

A plant experiment was made in which approximately seven gross of new quart bottles of one make were washed in a triple tank, 16 pocket-wide Meyer-Dumore washer, on each of 15 consecutive days. After the first time through, the whole lot was sampled at the discharge end by removing 2 bottles from every row of 16. These were thermal shock tested in the laboratory for comparison and are discussed under Part I (Lot B). The remainder of the bottles, numbering 868, were allowed to run to the fillers where they were loaded into new wood-post cases and subsequently moved by conveyors and 4 wheel trucks to the loading end of the washer. There

TABLE 4  
Data on washer experiment

Washing No.	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	Avg.
Temp. (°F.) of:																
Prerinses . . . . .	83	83	87	83	88	90	83	83	79	93	81	78	95	95	82	85.5
Tank No. 1 . . . . .	117	118	118	117	114	116	121	115	115	114	113	119	119	124	116	117.1
Tank No. 2 . . . . .	147	148	144	144	136	145	148	133	135	139	139	147	134	145	153	142.4
Tank No. 3 . . . . .	135	127	124	124	130	127	127	125	118	126	122	138	121	127	129	126.6
Outside brush spray . .	90	82	82	96	85	80	79	95	98	101	98	95	96	79	98	90.2
Inside spray . . . . .	91	91	83	77	87	87	86	96	81	90	88	85	81	92	83	86.5
Inside brush spray . .	91	91	83	77	87	87	86	96	81	90	88	85	81	92	83	86.5
City water spray . . .	60	60	60	60	61	61	61	62	62	63	63	62	62	62	64	61.6
Chlorine water spray .	63	63	63	63	64	64	64	65	65	65	65	65	65	65	67	64.6
Stops during washing in minutes . . . . .	0	0	12	0	3	0	0	0	0	6	0	2	0	0	0	
Thermal cracks—																
Cumulative per cent	1.3	1.8	2.5	2.5	2.9	2.9	3.0	3.1	3.1	3.5	3.5	3.7	3.8	3.9	3.9	

they were individually examined under a 150 watt daylight lamp and thermal and impact damages were noted. This individual examination was made after each washing. During the washings, temperatures were taken with accurate thermometers at the washer and all stops were noted. When the thermal shock cracks were very fine or just starting they were allowed to remain in the test in order to observe the further development of the cracks. Those with large cracks and impact shock defects were removed after each examination. Account was taken of these in the calculation of the cumulative percentage cracked by thermal shock.

The pertinent data collected in this experiment are shown in Table 4 in comparison with the cumulative percentage of bottles cracked by thermal shock. A good correlation was found between the stopping of the machine and thermal shock cracking. A satisfactory explanation of this relation has not yet been found, but its importance must be stressed. The thermal differential of the various shocks encountered by the bottles during the washing experiment are shown in Table 5. The greatest thermal

TABLE 5  
*Thermal differentials of shocks in washer during experiment*

Location of shocks	Thermal differential in °F.		
	Maximum	Minimum	Average
Prerinse to Tank No. 1 . . . . .	+41	+21	+21.6
Tank No. 1 to Tank No. 2 . . . . .	+37	+21	+25.3
Tank No. 2 to Tank No. 3 . . . . .	-24	-6	-15.8
Tank No. 3 to outside brush . . . . .	-48	-20	-36.4
Outside brush jet to inside jet . . . . .	-19	+1	-3.7
Inside jet to inside brush jet . . . . .	0	0	0.0
Inside brush jet to city water jet . . . . .	-34	-17	-24.9
City water jet to chlorine water jet . . . . .	+3	+3	+3.0

Note: Prerinse jet sprays inside of bottle; Tanks No. 1, 2, and 3 submerge bottles; city water jet sprays inside; and chlorine water jet sprays inside.

differential occurred at the outside brush spray. In the washings where cracking was greatest this differential was around 42°F. to 45°F.; although in one washing with high crackage (10th), this differential was only 25°F. At the outside brushes the bottles are pushed up between two rotating fiber brushes which are being continually sprayed with water at the indicated temperature. This is an outside-surface shock as contrasted to the double surface shocks in the laboratory tests. Preliminary tests in the laboratory have shown that when bottles are tested with the shock on the outside, *i.e.*, hot empty bottles immersed in cold water just up to the lip, a 10°F. lower differential has about the same effect as when the same bottles are tested by the double-surface shock. The failure of this type of test, however, to accurately shock the lip region has already been suggested.

## SUMMARY

N 1. The use of thermal shock tests is discussed and a suitable test for milk bottles is described.

2. The percentage of bottles cracked (up to 50%) as a function of the thermal differential was determined on two lots of bottles.

3. Repeated thermal shocking of bottles at a given temperature differential in laboratory tests, and repeated washings of bottles from the same lot in a commercial washer, both resulted in continual cracking with successive shocks.

4. Washings during which the machine stopped, resulted in more thermal shock cracking than washings during which the machine ran continuously.

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# NUTRIENTS FOR LACTATION, WORKING MAINTENANCE, AND GAIN IN LIVE WEIGHT IN AMERICAN DAIRY COWS

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This note supplements a previous paper (1) concerned with the evaluation of the working maintenance exponent,  $c$ , in the equation,  $DN'' = bW^c$ , notation<sup>1</sup> as before. A different method of mathematical analysis is applied to the same experimental data, as follows.

## METHOD OF ANALYSIS, ALL DATA

Distinction is made, as before, according to sign of  $\Delta W$ , that is, whether the cow gained ( $+\Delta W$ ) or lost ( $-\Delta W$ ) in live weight, on the average, during the experimental period. The records are arranged in increasing order by  $W$ , and divided into groups by successive 10's. Each group of records so formed is fitted with the equation,

$$DN = aFCM + K + d\Delta W \quad (1)$$

by use of the normal equations:

$$(n)K + (\sum FCM)a + (\sum \Delta W)d = \sum DN.$$

$$(\sum FCM)K + (\sum FCM^2)a + (\sum FCM\Delta W)d = \sum FCM DN.$$

$$(\sum \Delta W)K + (\sum FCM\Delta W)a + [\sum (\Delta W)^2]d = \sum \Delta W DN.$$

Each  $K$  is taken to represent  $DN''$ , or  $bW^c$ , for its group, and  $W$  is taken to be the average live weight shown by the 10 records of its group. The  $K$ 's and  $W$ 's thus derived are fitted with the equation,  $K = bW^c$ , by use of the weighted<sup>2</sup> normal equations:

$$(\sum K^2)\log b + (\sum K^2 \log W)c = \sum K^2 \log K.$$

$$(\sum K^2 \log W)\log b + [\sum K^2 (\log W)^2]c = \sum K^2 \log W \log K.$$

Finally, for a given set of  $K$ 's and  $W$ 's,  $a$  is taken to be the average of the  $a$ 's in that set; and similarly for  $d$ . We thus arrive at a solution of the equation,

$$DN = aFCM + bW^c + d\Delta W \quad (2).$$

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<sup>1</sup> Symbols are used to apply to each experimental period for each cow, as follows:

$DN$  = digestible nutrients intake, pounds per day

$DN'$  = digestible nutrients apportioned to lactation, pounds

$DN''$  = digestible nutrients apportioned to maintenance, pounds per day

$DN'''$  = digestible nutrients apportioned to gain in weight, pounds

$FCM$  = milk-energy yield, pounds of 4 per cent milk per day

$W$  = average live weight, pounds

$\Delta W$  = average gain in live weight, pounds per day

$n$  = number of cows or records

$D$  = observed  $DN$  - calculated  $DN$ .

<sup>2</sup> Dr. W. Edwards Deming, U. S. Department of Agriculture, has very kindly called my attention to the necessity of weighting the normal equations by  $K^2$ . Neither the weighted nor unweighted normal equations give a true least-squares fit for  $K = bW^c$  when the observed  $K$ 's are so highly irregular as they are in the present data.

In equation (2),  $a$  is the pounds of digestible nutrients per pound of FCM apportioned to lactation;  $b$  is the pounds of digestible nutrients per day per pound of live weight raised to the power,  $c$ , apportioned to working maintenance; and  $d$  is the pounds of digestible nutrients per pounds of gain in live weight, apportioned to gain in live weight. Or, in symbols,  $DN' = aFCM$ ,  $DN'' = bW^c$ ,  $DN''' = d\Delta W$ , and  $DN = DN' + DN'' + DN'''$ .

A positive  $d$  means that the method apportions a consumption of nutrients for gain in live weight or a release of nutrients for loss in live weight. A negative  $d$  means a consumption of nutrients for loss in weight and a release of nutrients for gain in weight. While a negative  $d$  is opposed to logical expectation, it is not at all contrary to a mathematical balancing of the values to which equation (1) is fitted, because of normal irregularities and errors in the observations.

TABLE 1

*Digestible nutrients apportioned to lactation, working maintenance, and live-weight gain*  
Records of Guernsey, Holstein and Jersey cows from various experiment station sources, in groups of 10.

(See footnote, page 585, for explanation of symbols.)

Group No.	Sign of $\Delta W$	n	Live-weight Limits, lbs.	W	$DN' = aFCM$ a	$DN'' = bW$ 1000 b	$DN''' = d\Delta W$ d
1	-	10	634-783	732	.2385	10.57	1.82
2	+	10	735-790	770	.2732	9.77	1.63
3	-	10	792-824	808	.3003	7.94	2.30
4	+	10	793-843	819	.4256	4.64	12.38
5	-	10	833-858	846	.2836	8.73	1.84
6	+	10	846-885	864	.1507	13.99	-.30
7	-	11	859-898	878	.3089	9.01	6.70
8	+	10	887-925	906	.2812	8.30	3.58
9	-	10	902-978	928	.2277	11.26	.28
10	+	10	929-996	970	.3219	8.99	1.35
11	+	10	1008-1072	1046	.3172	7.87	2.80
12	+	10	1073-1120	1096	.2927	9.02	1.53
13	-	10	985-1195	1105	.3031	8.27	-2.65
14	+	10	1128-1172	1152	.2519	10.58	-1.15
15	+	12	1175-1203	1188	.3290	8.06	.10
16	+	10	1207-1232	1220	.3170	7.32	1.34
17	-	10	1203-1273	1242	.4083	4.74	-2.88
18	+	10	1239-1267	1251	.2702	8.59	2.76
19	+	10	1271-1287	1278	.2434	9.22	1.37
20	+	10	1288-1301	1296	.0906	13.34	.79
21	+	10	1305-1323	1314	.3185	6.86	1.83
22	+	10	1327-1341	1335	.2776	7.80	3.33
23	+	10	1341-1382	1363	.1846	10.04	.71
24	-	10	1331-1500	1395	.2712	8.27	1.82
25	+	10	1384-1453	1402	.1815	10.72	-2.50

## Results, All Data

The 172  $+\Delta W$  records give,  $DN = .266FCM + .0134W^{.96} + 1.86\Delta W$ .

The 81  $-\Delta W$  records give,  $DN = .293FCM + .1976W^{.55} + 1.15\Delta W$ .

Both groups together give,  $DN = .275FCM + .0161W^{.93} + 1.63\Delta W$ .

The equation for both groups together is derived from the data of Table 1, in which the figures in the  $DN''$  column are equal to  $1000K$  of equation (1) divided by  $W$  of Table 1. That is, the  $+\Delta W$  and  $-\Delta W$  groups are allowed to keep their identity, rather than to mix  $+\Delta W$  and  $-\Delta W$  records in the same group. This may or may not be necessary. It is done with the thought that it may give more representative values to the  $K$ 's of equations (1).

If the above result for both groups is taken as a summary of the 253 records, it appears that working maintenance is proportional to the .93 power of live weight. What does this mean from the standpoint of a practical feeding standard for cows in milk? The  $DN''$  column of Table 1 gives, as indicated, the nutrients for working maintenance,  $K$  of equation (1), per day per 1000 pounds live weight, for each of the 25 groups of 10 records. In Figure 1 working maintenance per 1000 pounds live weight is plotted against live weight. The correlation between the two is  $r = -.07 \pm .15$ . The regression equation is  $1000DN''/W = 9.67 - (.0007 \pm .0014)W$ .

As thus determined, working maintenance per unit live weight is quite variable, as may be seen in Figure 1. There is a tendency for it to decrease

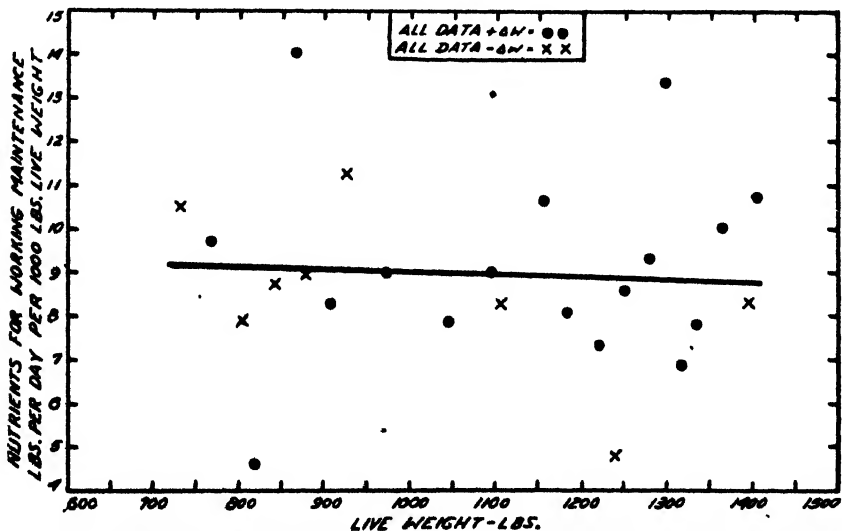


FIG. 1. Relation of working maintenance per unit live weight to live weight, from Table 1.

with live weight, but this tendency is not statistically or practically significant.

## Minnesota and Cornell Data

The present method of analysis requires a considerable number of records to be justifiable. It is permissible, however, to apply it to the Minnesota data ( $n = 98$ ) and to the Cornell data ( $n = 103$ ). The same general plan is followed, using groups of successive 5's. Tables 2 and 3 give the results for the groups of 5. In Figure 2 working maintenance per 1000 pounds live

TABLE 2

*Digestible nutrients apportioned to lactation, working maintenance and live-weight gain*  
Records of Guernsey, Holstein and Jersey cows from Minnesota Experiment Station, in groups of 5.

(See footnote, page 585, for explanation of symbols.)

Group No.	Sign of $\Delta W$	n	Live-weight Limits, lbs.	W	$DN' = aFCM$ a	$DN'' = bW$ 1000 b	$DN''' = d\Delta W$ d
1	-	5	634- 735	698	.1910	10.87	- 8.13
2	+	4	735- 765	753	.3452	8.27	2.94
3	-	5	752- 783	765	.3490	7.59	4.17
4	+	5	766- 789	780	.4353	6.25	-10.15
5	-	5	792- 809	800	.2163	9.15	-1.64
6	-	10*	811- 884	827	.3361	7.11	3.39
7	+	15*	790- 887	840	.0544	16.76	- 4.31
8	-	5	845- 858	853	.3640	6.82	1.12
9	-	5	859- 877	869	.5267	2.89	10.82
10	-	5	887- 898	890	.3266	9.46	8.75
11	-	5	902- 923	912	.2897	8.83	1.07
12	+	5	890- 976	933	.2840	8.44	12.71
13	-	5	925- 978	944	.2578	9.56	-10.94
14	+	5	978-1071	1024	.5004	6.35	-14.96
15	-	5	985-1273	1073	.3109	8.20	.61
16	+	9*	1072-1315	1177	.1999	11.53	- 2.04

\* These combinations were made to get rid of negative values of b, appearing in the groups of 5.

weight is plotted against live weight for the Minnesota and Cornell data separately.

The correlations between working maintenance per unit live weight and live weight are, Minnesota,  $r = .06 \pm .17$ , and Cornell,  $r = .03 \pm .15$ . The regression equations are, Minnesota,  $1000DN''/W = 7.38 + (.0014 \pm .0042)W$ , and, Cornell,  $1000 DN''/W = 7.95 + (.0009 \pm .0043)W$ . Both the Minnesota and Cornell experiments show a tendency for working maintenance per unit live weight to increase with live weight, but in neither case is this tendency statistically or practically significant.<sup>3</sup>

<sup>3</sup> It will be apparent that correlating  $K/W$  with  $W$  from equation (1) is essentially a test of the postulate that working maintenance is proportional to live weight. In a similar way, to test the postulate that working maintenance is proportional to physiologic weight, which is proportional to the .73 power of live weight, as contended by Brody (2), we would correlate  $K/W^{.73}$  with  $W^{.73}$ . This gives, from the 25 groups of Table 1, all

TABLE 3

*Digestible nutrients apportioned to lactation, working maintenance and live-weight gain*  
 Records of Holstein cows from Cornell Experiment Station, in groups of 5.  
 (See footnote, page 585, for explanation of symbols.)

Group No.	Sign of $\Delta W^*$	n	Live-weight Limits, lbs.	W	$DN' = aFCM_a$	$DN'' = bW_{1000\ b}$	$DN''' = d\Delta W_d$
1	+	5	1081-1159	1126	.2815	9.05	-1.02
2	+	5	1163-1181	1171	.2607	10.19	-1.02
3	-	5	1155-1195	1173	.3047	8.30	-1.14
4	+	5	1184-1196	1191	.2460	9.63	2.61
5	+	5	1203-1220	1213	.2691	8.27	3.54
6	-	5	1203-1238	1224	.4188	4.48	-3.56
7	+	5	1220-1232	1226	.3925	5.62	1.28
8	+	5	1230-1251	1245	.0865	13.55	-.30
9	+	5	1266-1272	1270	.2395	9.86	-.74
10	-	5	1251-1331	1272	.3222	7.14	-.09
11	+	5	1272-1285	1278	.2358	8.85	3.69
12	+	5	1286-1296	1291	.2143	10.19	-.64
13	+	5	1297-1305	1300	.0457	15.11	-1.13
14	+	5	1306-1323	1314	.2564	9.21	-.75
15	+	5	1323-1333	1328	.2616	8.47	2.05
16**	+	5	1337-1344	1340	-.2435	21.87	-6.98
17	-	5	1332-1379	1355	.1959	10.04	3.86
18	+	5	1346-1375	1364	.2260	8.85	1.76
19	+	5	1382-1389	1386	.2183	9.11	1.76
20	+	4	1398-1453	1423	.3419	6.77	-2.09
21	-	4	1394-1500	1454	.2124	9.25	.91

\* There are here 79 +  $\Delta W$ 's and 24 -  $\Delta W$ 's, instead of 80 and 23, respectively, in the previous paper (1). This is due to working directly with Cornell Bulletins 540 and 578, thus eliminating several errors in Missouri Research Bulletin 239. These errors are still present in Table 1.

\*\* This group is excluded in figure 2 and in the computation of correlation, etc., on account of the negative value of  $a$ . It was found impossible to get rid of this negative value by including an adjoining group, as in Table 2.

data,  $r = .17 \pm .13$ ; from the 16 groups of Table 2, Minnesota data,  $r = .17 \pm .17$ ; from the 20 groups of Table 3, Cornell data  $r = .10 \pm .15$ . From the 36 groups of Tables 2 and 3, Minnesota and Cornell data together,  $r = .29 \pm .10$ , a result which borders on statistically significant evidence that the  $W^{.73}$  postulate is not supported by the experimental results. Regardless of statistical significance, indicated by the "probable errors," the best estimate from the Minnesota and Cornell data, together or separately, is that working maintenance per unit live weight tends to increase with live weight. These two sets of data are given emphasis because they agree with each other (see Figure 2), and each in itself possesses a homogeneity of experimental conditions which may be lacking when the other 52 records from miscellaneous sources are included.

A point of interest is the relation between live weight and nutrients for lactation per pound of FCM, that is: the correlation between  $W$  and  $a$  of Tables 1, 2 and 3. The coefficients are, from the 25 groups of Table 1, all data,  $r = -.20 \pm .13$ ; from the 16 groups of Table 2, Minnesota data,  $r = .01 \pm .17$ ; from the 20 groups of Table 3, Cornell data,  $r = -.22 \pm .15$ .

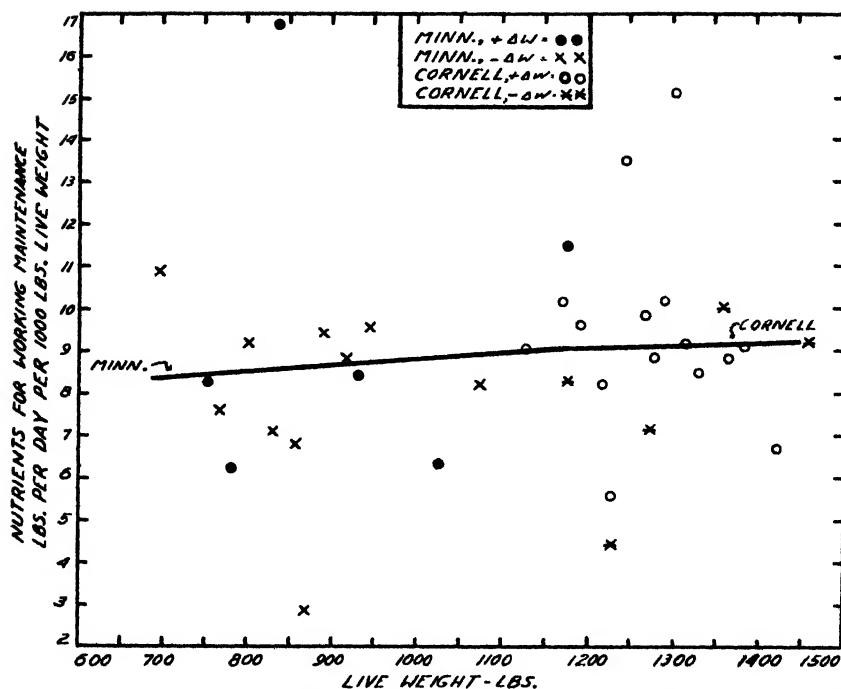


FIG. 2. Relation of working maintenance per unit live weight to live weight, from Tables 2 and 3.

#### DISCUSSION

It is amazing that, under the present treatment, the Cornell data show working maintenance to be proportional to live weight (or to a power of live weight slightly greater than unity), while under the former treatment (1) the same data showed working maintenance to be proportional to the .15 power of live weight, or substantially independent of live weight. In the former treatment the problem of nutrients for gain in weight,  $\overline{DN}'' = d\Delta W$ , was summarily disposed of by assigning values to  $d$  according to the proposal of Knott, Hodgson and Ellington (3), that is,  $d = 3.53$  for  $+\Delta W$ 's and  $d = 2.73$  for  $-\Delta W$ 's. Could this summary procedure be responsible for the great difference in result?

For answer, the Cornell data have been reworked under the former plan but including  $\Delta W$  in the normal equations, thus allowing  $d$  to find its own value from the observations themselves. Following is a comparison of the former results (1),  $\Sigma D^2/(n-2)$ , as against the inclusion of  $\Delta W$ ,  $\Sigma D^2/(n-3)$ , using  $(n-3)$  since we now have 3 constants in the equation as fitted:

Trial value of $c$	$= 0$	.15	.45	.73	1.00	1.27	2.00
$\Sigma D^2/(n-2), 80 + \Delta W$ 's	.832	.818	.836	.907	1.017	1.159	1.628
$\Sigma D^2/(n-3), 80 + \Delta W$ 's	.964	.637	.318	.252	.417	1.154	1.824
$\Sigma D^2/(n-2), 23 - \Delta W$ 's	.442	.440	.548	.781	1.102	1.481	2.501
$\Sigma D^2/(n-3), 23 - \Delta W$ 's	.356	.364	.492	.743	1.082	1.474	2.513

Inclusion of  $\Delta W$  in the normal equations makes a large difference in the  $+\Delta W$  records, shifting  $c$  of equation (2) from .15 to .6 (that is, between .45 and .73). Inclusion of  $\Delta W$  makes little difference in the  $-\Delta W$  records, shifting  $c$  from .15 to zero, or slightly less. Evidently the treatment of  $\Delta W$  in the previous paper does not explain the difference in result as compared with the present paper. In equation (1)  $K$  becomes a sort of average value of working maintenance (although including all items other than FCM and  $\Delta W$ ) for the group. It seems worth while to apply the trial-value-of- $c$  method of fitting equation (2) to the average DN, FCM,  $W$  and  $\Delta W$  values of the groups in Table 3. This leads to the result,  $c = .15$ , that is, not different than previously obtained (1) from the individual records. The trial-value-of- $c$  method amounts to a (clumsy) simultaneous solution for  $a$ ,  $b$ ,  $c$  and  $d$  of equation (2) and it is hard to see why it should not give right results in solving equation (2). However, since in practice it so frequently leads to nonsensical results, it should be discarded in favor of the present method of solving equation (2) through equation (1), where suitable observations are available.

Suitability of the observations depends not only on a sufficiency with respect to numbers, but also on adequacy with respect to design of the experiments. The experiments here utilized were not designed to determine the amount of nutrients used for lactation, on the one hand, and for maintenance, on the other. Thus, a major problem has been to learn the effect of varying proportions of protein in the ration, and there is the possibility that these variations may impair the value of the data for the purpose here used. In view of the great practical and theoretical importance of knowing the amount of nutrients required for lactation and the amount required for working maintenance it would be desirable to carry out experiments<sup>4</sup> designed for the purpose.

The Minnesota and Cornell feeding trials here utilized were largely guided by a standard assigning nutrients for working maintenance as proportional to live weight. Can this be responsible for the outcome pictured in Figure 2, indicating that working maintenance is proportional to live weight? Evidently not, at least we shall see in a later paper (to appear in the October issue of this Journal) that the direct proportionality holds in a

<sup>4</sup> The problem and its solution by the partition-equation method have been beautifully presented by Brody and Cunningham (4). A pedometer record of distance of travel and a record of time lying and standing would be valuable additions to the record of live weight, in the working maintenance problem. A fault of unknown influence in the present treatment is the assumption that live weight follows a strictly linear course from start to finish of the trial, whereas it is known that live weight normally follows a decidedly curvilinear course through the lactation period. That portion of the live-weight curve which is substantially horizontal should be especially suited to the equation method of evaluating the nutrient requirements for lactation and maintenance. Any long trial could well be broken up into short periods to give segments of increasing, decreasing and stationary live weights.

large body of Danish records, although the cows were fed to a working maintenance standard approximately proportional to the  $2/3$  power of live weight (or, substantially, in units of this paper,  $DN'' = .0645 W^{2/3}$ ).

The theory that maintenance is proportional to the  $2/3$  power of live weight has been a favorite for the reason that surface area and consequently amount of heat loss, vary as the  $2/3$  power of weight. An inactive dry cow may have to expend energy to keep warm, and to that extent idle maintenance may vary directly with  $W^{2/3}$ . An active milking cow may have to expend energy to keep cool, and to that extent working maintenance may vary inversely (instead of directly) with  $W^{2/3}$ . Working maintenance bears a very different relation to live weight than does idle maintenance, or especially, "basal" metabolism. We are here concerned with working maintenance of milking dairy cows, and this can be determined only under conditions of work in milk production.

#### CONCLUSION

It is concluded from the above results that for cows of the Guernsey, Holstein and Jersey breeds, under conditions of the experiments, a proper feeding standard is  $DN = .275FCM + .009W$ , where  $DN$  is pounds of digestible nutrients per day,  $FCM$  is milk-energy yield in terms of pounds of 4 per cent milk per day, and  $W$  is live weight in pounds.

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## RIBOFLAVIN CONTENT OF MILK COLLECTED IN DIFFERENT MONTHS AND CORRELATED WITH OTHER CONSTITUENTS OF THE MILK<sup>1</sup>

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Interest in variations of the riboflavin content of milk is a natural consequence of previous indications that d-ribose may be isolated from whey (10). The availability of a rapid method for the determination of riboflavin in milk (13) offers opportunity for extensive studies of the amount of this substance in milk. Biological determinations at this station (7) have shown colostrum to be richer in flavin than milk produced later in lactation. Such determinations have also suggested that the amounts of riboflavin in samples from groups of different breeds on widely different rations vary within a comparatively narrow range. The purpose of this study was to apply the rapid method of riboflavin determination to samples of normal milk collected at intervals from individual cows of different breeds on different rations; and to compare these values with the seasons and the cows involved; and with the concentrations of various other constituents in the milk.

### PROCEDURE

Samples were procured from each milking of each cow in the station herd on 3 successive days in March, April, May, and July.<sup>2</sup>

The animals used included 4 breeds. A group of young Holsteins on a special ration which had never included pasture, and a group including all breeds which for two periods of six weeks each was fed a ration low in carotene, were also studied. The regular ration included Atlas sorgo silage, alfalfa hay, and a grain mixture of yellow corn, bran, and cotton seed meal. The ration for the experimental Holstein group differed in that white corn was used in the grain mixture and that prairie hay replaced the alfalfa, or for some cows, both the alfalfa and silage. During the time the flavin tests were made, the low carotene ration included wheat straw, cotton seed meal

<sup>1</sup> Contribution No. 227, Department of Chemistry, and No. 78, Department of Food Economics and Nutrition.

<sup>2</sup> The samples were supplied by Professor H. W. Cave of the Department of Dairy Husbandry and were used for several projects in which the Departments of Chemistry and Dairy Husbandry were cooperating.

and molasses. Earlier in the year a grain mixture of yellow corn and bran had been fed the more heavily producing cows of this group. Shortly before the May samples were collected, limited amounts of pasture were provided for all cows except the experimental Holsteins. Pasture later became abundant so that all except the experimental Holsteins received a generous pasture supplement before the July samples were collected.

The determinations for flavin (13), vitamin C (14), and phosphatase (6) were made on morning milks only and on the days the samples were collected. The determinations for total solids<sup>3</sup> (1), fat<sup>3</sup> (1-Babcock), fat color (11), lecithin (4), protein chlorine<sup>3</sup> (8), and lactose (1) were made on composites for each cow of each milking of each 3-day period. The value for protein was obtained by subtracting from the total solids, the sum of the measured values for fat and lactose and an assumed value of 0.75 per cent for ash. All fluorimetric readings for flavin were made jointly by 2 or sometimes 3 observers. The microscopic tests<sup>4</sup> for leucocytes, and for mastitis streptococci, and other forms of bacteria were made on samples of fore milk from small groups of cows at intervals of 2 to 4 weeks. The method of calculating mastitis scores from these tests is described elsewhere (12). The results of these microscopic tests were interpolated to the times at which chemical analyses were made. Comparisons<sup>5</sup> of flavin with breeds, seasons, mastitis, and constituents measured on 3-day composite samples were based on 3-day average values for flavin. Comparisons with constituents measured daily were on the basis of daily values.

## RESULTS

The relations of flavin to breeds, stages of lactation, and seasons are shown in tables 1 to 3 respectively. The flavin content of milk from none of the straw fed cows appeared unusual for the breed concerned, or in relation to later values on the same cow. Values for these milks were, therefore, included with the milk from normally fed cows. The average flavin content of milk increased significantly from Ayrshires to Holsteins, from Holsteins to Guernseys, and from Guernseys to Jerseys. (See table 1.) While the experimental Holsteins produced milk with slightly more flavin than the regular Holsteins, this difference is probably not significant.

The average differences in flavin content of milk samples collected from any one breed of cows, either  $\frac{1}{2}$  to 5 months, or 5 to 10 months after the cows

<sup>3</sup> These determinations were made by Mr. W. J. Caulfield of the Department of Dairy Husbandry.

<sup>4</sup> These tests were made by Dr. A. C. Fay and Mr. V. D. Foltz of the Department of Bacteriology as part of a project in which they were cooperating with the Department of Dairy Husbandry.

<sup>5</sup> The authors acknowledge the assistance of (a) Mr. H. H. Laude of the station staff, and Prof. M. C. Moggie of Kansas State College, (b) Dr. George W. Snedecor of the Statistical Laboratory of Iowa State College in making these comparisons.

TABLE 1

*Flavin content of milk from different breeds of cows*

Breed	No. of 3-day periods Mar., Apr., May	Flavin content p. p. m.	S.E. of means
Experimental Holsteins	41	1.43	.053
Ayrshires	34	1.17	.036
Regular Holsteins	45	1.37	.043
Guernseys	38	1.53	.048
Jerseys	47	1.73	.042

TABLE 2

*Flavin content of milk in relation to stage of lactation by breeds*

Breed	Cows No.	Tests No.	State of lactation months	Flavin content	
				Cows p. p. m.	Tests p. p. m.
Experimental Holsteins	9	21	$\frac{1}{2}$ to 5	1.24	1.45
	4	10	5 " 10	1.35	1.35
Regular Holsteins	11	16	$\frac{1}{2}$ to 5	1.29	1.39
	8	20	5 " 10	1.38	1.38
Ayrshires	8	17	$\frac{1}{2}$ to 5	1.16	1.16
	6	16	5 " 10	1.16	1.15
Guernseys	4	10	$\frac{1}{2}$ to 5	1.54	1.49
	7	23	5 " 10	1.51	1.53
Jerseys	10	12	$\frac{1}{2}$ to 5	1.60	1.57
	11	31	5 " 10	1.58	1.78

freshened, were so small as to be considered negligible. Averages for each stage of lactation in each group of cows were calculated both on the basis of single tests, and also on the basis of averages for each cow in the stage and group. On either basis the flavin content of milk was higher in the first stage of some groups and in the last stage of others.

Although the mean flavin content of milk increased for all the breeds from May to July (see table 3) it is doubtful whether this increase should be ascribed to the change in rations. If pasture produced the increase, it should have started in May. For Guernseys there was in May an increase of some significance, while the experimental Holsteins were the only herd to show a significant decrease at this time. The changes in the other three breed groups were very small. It might be argued that a longer time was needed for pasture to produce the expected rise in flavin. The July values indicated significant rises for only the Guernseys and experimental Holsteins. The flavin increased much more for the experimental Holsteins that received no pasture, than for any other group except the small herd of Guernseys.

The coefficients of correlation between concentrations of flavin in the

TABLE 3

*Changes in the riboflavin content of milk produced in different months*

Cows used as sources of milk	Date of collection of samples	Average change in flavin p. p. m.	Critical* ratio of change	Coefficients of correlation
15 Experimental	Mar. and Apr.	-0.23	1.51	+0.18
11 Holsteins	Apr. " May	-0.12	3.6	+0.85
9	May " July	+0.23	2.8	+0.07
11 Regular	Mar. and Apr.	-0.07	1.05	+0.64
15 Holsteins	Apr. " May	+0.06	0.95	+0.68
11	May " July	+0.02	0.17	+0.57
9 Ayrshires	Mar. and Apr.	-0.09	0.77	-0.40
10	Apr. " May	-0.00	0.04	-0.48
9	May " July	+0.04	0.51	-0.35
12 Guernseys	Mar. and Apr.	-0.07	0.39	-0.03
12	Apr. " May	+0.25	2.2	+0.00
6	May " July	+0.18	4.6	+0.87
16 Jerseys	Mar. and Apr.	+0.02	0.24	+0.45
15	Apr. " May	+0.05	1.00	+0.49
10	May " July	+0.15	1.2	+0.58

$$\sqrt{\frac{s^2x + s^2y - 2sxsy}{N}}$$

See:

- G. U. Yule, Introduction to Theory of Statistics Ed. 5 p. 211.  
 Griffith.  
 G. W. Snedecor, Statistical Methods. p. 132. Collegiate Press,  
 Ames, Ia.  
 G. E. Garrett, Statistics in Psychology and Education. p. 287.  
 Longmans.

milk of given cows on successive months furnish an important measure of the significance of the changes in average values (14). The large variations in these correlations (see last column of table 3) indicate that the causes of variation were not uniform. The variations of climate and ration, which were uniform for all cows were, therefore, certainly not the only important factors which caused the variations in the flavin content of the milk. The Guernseys and Ayrshire breeds showed more irregular correlations, and less consistent relations of flavin to fat than the other three groups.

The relation of flavin to milk yield was found very variable and a helpful discussion of this relation must await further study. Relations of the flavin content of milk to the concentrations of 12 other constituents in the milk are shown in table 4.

The fat is the only one of these constituents for which there was anywhere near a consistent, significant relation in the total group of comparisons made. Effects of season, age of cow, stage of lactation, etc., may later be found to have some significant relations. These effects were found to be unimportant for the relation between flavin and fat. Average values of these constituents for each cow are shown in figure I. In each group except Guernseys there appears to have been a significant positive correlation be-

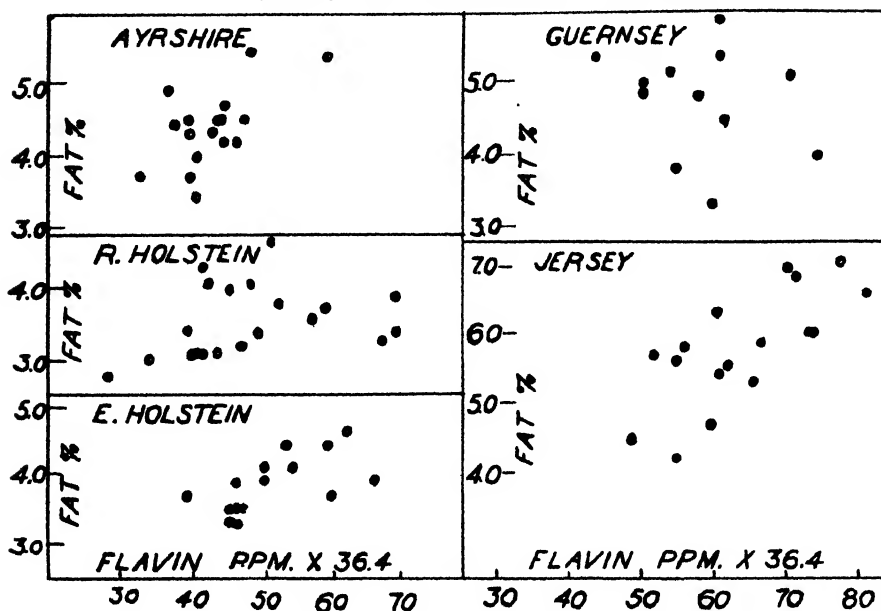
TABLE 4  
Relations of flavin to other constituents of milk in spring and summer months

Breed	Vitamin C per cent of milk				Loss of C. 72 hrs. at 4° C.				Carotene per cent of fat				Lecithin per cent of fat			
	N	r	CR*		N	r	CR		N	r	CR		N	r	CR	
				3, 4, 5/37				3/37				3, 4, 5/37				3/37
Experimental Holsteins	114	-.025	.26		28	-.314	1.69		35	-.203	1.20		11	-.248		
Regular Holsteins	125	-.145	1.63		36	-.305	3.41		45	-.145	.98		11	-.092		
Ayrshires	96	-.064	.63		36	-.134	.80		33	-.088	.50		11	-.018		
Guernseys	98	-.063	.67		31	-.132	.73		38	-.286	1.80		13	-.483		
Jerseys	136	-.403	5.10		46	-.121	.82		53	-.160	1.68		15	-.166		
				Fat				Protein				Lactose				Phosphatase
				2, 4, 5, 7/37				3, 5, 7/37				3, 5, 7/37				3, 4, 5/37
Experimental Holsteins	51	-.404	3.65		35	-.210	1.25		36	-.014	.08		103	-.048	.49	
Regular Holsteins	59	-.352	2.80		41	-.034	.22		44	-.016	.10		116	-.148	1.82	
Ayrshires	44	-.437	3.15		35	-.118	.69		37	-.110	.67		92	-.206	2.02	
Guernseys	40	-.240	1.55		31	-.199	1.12		32	-.013	.07		95	-.354	3.65	
Jerseys	57	-.490	4.15		38	-.246	1.59		38	-.231	1.39		127	-.250	2.91	
				Leucocytes				Streptococci				Odd bacteria				Chlorine
				3, 4, 5/37				3, 4, 5/37				3, 4, 5/37				3, 4, 5, 7/37
Experimental Holsteins	39	-.220	1.38		39				39	-.161	1.00		51	-.060		
Regular Holsteins	45	-.242	1.63		45	-.328	2.20		45	-.230	1.55		59	-.160		
Ayrshires	33	-.354	3.70		33	-.245	1.41		33	-.296	1.73		44	-.030		
Guernseys	38	-.109	.67		38	-.105	.64		38	-.090	.55		40	-.116		
Jerseys	47	-.096	.66		47	-.435	3.20		47	-.190	1.32		58	-.180		

$$* CR = \frac{r(N-2)^2}{(1-r^2)^2}$$

George W. Snedecor, Statistical Methods 1937 Edition, Page 125, Collegiate Press Inc., Ames, Iowa.

FIGURE 1 FLAVIN AND FAT IN MILK FROM COWS OF DIFFERENT BREEDS



tween flavin and fat content. The apparent negative relation for Guernseys may be due to the small number of cows tested. If real, it would be a more serious exception to the relation in other groups if these Guernseys were not also exceptional in the relations of lecithin, and phosphatase to flavin. The authors have at present no explanation for this possible unusual behavior of the Guernsey group, but the distinctive relation of flavin to three different constituents, indicates considerable probability that either Guernseys as a breed, or this particular group, were unusual.

That the fat, which is in an entirely different phase of the milk from the flavin, was the only constituent to which the flavin was regularly related is indeed surprising. There is, however, evidence that flavin functions in combination with protein (9), and that milk-fat is synthesized from carbohydrates derived from proteins or amino acids (5). If flavin plays a rôle in fat synthesis, it would not be surprising that the disturbance of this rôle among Guernsey cows was associated with unusual relations of flavin to lecithin and phosphatase. Both the flavin and phosphatase values were subject to large daily variations. While some of the variations in fat and lecithin were lost in the use of composite samples, it is generally accepted that fat, at least, is also one of the most variable constituents of milk. The variation of lecithin is less known, but from the values summarized in table 4 and from other values on samples for which flavin was not measured (2, 4), we may expect this variation to be as great as for fat. Little or nothing

is known of the rôle played by either flavin, phosphatase or lecithin in the synthesis of milk fat. They would all be classed among the active or unstable constituents of milk and might therefore be involved in this synthesis.

#### SUMMARY

Riboflavin determinations were made on samples of milk collected at intervals from individual normal cows of different breeds and on different rations. The influences of season and individuality on flavin values were considered. The relationship of flavin content to the concentrations of the following constituents in the milk were tested: vitamin C, loss of C on 72 hours storage at 4° C, carotene, lecithin, fat, protein, phosphatase, leucocytes, mastitis streptococci, odd bacteria, lactose, and chlorine.

The average flavin contents in March, April, and May were for Ayrshires 1.17 parts per million, for Holsteins 1.37, for Guernseys 1.53, and for Jerseys 1.73. Milk from cows between 15 days and 10 months after freshening, showed no significant difference in flavin content.

The flavin content of milk was slightly higher in July after the cows had been pastured. The climate and ration were not the only important factors which caused variations in the flavin content of milk.

The fat is the only constituent of milk for which an approach to a significant consistent relation to flavin was found. It is suggested that flavin may have a rôle in the production of milk fat.

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# BOUND WATER AND ITS RELATION TO SOME DAIRY PRODUCTS

## III. THE RELATION OF BOUND WATER TO SOME DAIRY PHENOMENA<sup>1</sup>

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Previous studies have shown that liquid dairy products contain appreciable amounts of bound water (1) and that the bound  $\rightleftharpoons$  free water equilibrium may be changed by certain treatments (2). Since milk and other dairy products contain a colloidal complex, it was thought desirable to investigate certain dairy phenomena from the standpoint of hydration.

### EXPERIMENTAL METHODS

The samples and methods used were, on the whole, as previously described (1). Although in certain experiments changes in samples and methods were made, these changes will be dealt with under the sections reporting the experimental work.

#### POSSIBLE RELATION OF BOUND WATER CONTENT TO THE CREAMING OF MILK

The gravity formation of a maximum cream layer on bottled milk is of considerable importance in the market milk industry, largely because of the layman's method of judging richness. The processor of milk knows in a general way the factors affecting creamline but does not understand exactly why heat treatment is destructive to creamline. It was to supplement the existing knowledge of the creaming of milk that this work was undertaken in the hope that further explanation would be obtained.

The inhibited creaming of heated milk has attracted attention ever since pasteurization was first accepted as a general practice in the milk industry and much has been written in attempting to explain this phenomenon. Rahn (3) showed that the creaming property can be restored after heating by the addition of gelatin or other accelerating colloids. Van Dam and Sirks (4) added to milk such colloids as gelatin, starch, Irish Moss and gum tragacanth and obtained a 15–25 per cent increase in volume of cream, and postulated that fat clumping took place. Babcock and Russell (5) and others found that the presence of fat clumps was essential to creaming.

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siderable importance in the creaming of milks of uniform fat content. They stated that the calcium caseinate was a factor in creaming, and thus it was affected by pasteurization temperatures. The whey colloids, which are greatly hydrated, are effective promoters of cream rising. These investigators state further that "Both exhaustiveness of rise of fat and greater volume of cream are promoted by the truly hydrophilic colloids and depressed by colloids of hydrophobic properties."

Therefore it might be that the effect of heat on creaming centers around the plasma colloids to a certain degree. The conditions and the forces acting at the fat/plasma interface may also be of importance. A truly hydrophilic colloid added to milk gives a quicker cream rise, a larger cream layer of lower fat content, and a plasma of lower fat content. The increase in viscosity of milk by non-colloids like sugar delays creaming (3).

The chief stability factor of a hydrophilic colloid is its degree of hydration. The fat globule membrane is composed chiefly of phospholipids and protein. That the phospholipids are greatly hydrophilic has been previously shown (1). It is also known that the casein of milk owes its stability to charge and hydration. With this information a series of experiments were made to determine the relationship between high heat treatment, bound water content, and the creaming ability of milk.

Three experiments were conducted as follows: (1) Raw whole milk was heated in a water bath to different temperatures and held for various lengths of time. (2) Raw cream was added to skimmilk which had been heated to various temperatures. (3) Raw skimmilk was added to cream which had previously been heated to various temperatures. In each case unheated samples were used as controls and all samples came from the same source. The temperatures used were 143° F. for 30 minutes, 160° F. for 5 minutes, and 180° F. momentarily. After the skimmilk or cream was heated, it was cooled to approximately 80° F. before reconstituting. Samples for creaming were stored at 38° F. in 100 ml. graduated cylinders and examined after 24-hour intervals. The results are expressed as percentage cream volume per one per cent fat.

The results of these experiments are recorded in Tables 1, 2 and 3. Table 1 shows the effect of heat on the creaming ability and bound water content of fresh whole milk. Table 2 shows the effect of heat on the creaming ability and bound water content of whole milk prepared from heated skimmilk to which fresh raw 48 per cent cream was later added. Table 3 shows the effect of heat on the creaming ability and bound water content of whole milk prepared from heated 54 per cent cream to which raw skimmilk was later added.

Pasteurization of whole milk at 143° F. for thirty minutes, if done correctly, has very little effect on the creaming ability of the milk. The bound water reduction was slight when compared to higher heat treatments. At

TABLE 1  
*The effect of heat treatment on the creaming ability and bound water content of fresh raw milk*

Treatment	Hours aged at 40° F.	Per cent solids	Alcohol No.	Viscosity centipoises	Volume cream layer per one per cent fat	Per cent bound water	Per cent decrease in bound water content due to heating
1. Unheated	24	11.92	8.1	2.204	4.23	3.75	-
2. Heated to 143° F. for 30 min.	24	12.09	9.1	2.102	4.06	3.56	5.6
3. " " 160° F. for 5 "	24	11.94	9.3	2.041	0.77	3.06	18.4
4. " " 180° F. for 0 "	24	12.11	9.3	2.061	0.76	3.30	12.0

TABLE 2  
*The effect of heat treatment on the creaming ability and bound water content of reconstituted milk (heated skim milk plus raw cream)*

Treatment	Hours aged at 40° F.	Per cent solids	Alcohol No.	Viscosity centipoises	Volume cream layer per one per cent fat	Per cent bound water	Per cent decrease in bound water content due to heating
1. Unheated	24	12.43	7.6	2.122	4.11	3.41	
2. Skim milk heated to 143° F. for 30 min.	24	12.19	8.0	2.061	3.00	3.11	8.8
3. Skim milk heated to 160° F. for 5 min.	24	12.24	8.3	2.241	0.72	2.45	28.1
4. Skim milk heated to 180° F. for 0 min.	24	12.38	8.3	2.306	0.64	2.29	32.8

TABLE 3  
*The effect of heat treatment on the creaming ability and bound water content of reconstituted milk (heated cream plus raw skim milk)*

Treatment	Hours aged at 40° F.	Per cent solids	Alcohol No.	Viscosity centipoises	Volume cream layer per one per cent fat	Per cent bound water	Per cent decrease in bound water content due to heating
1. Unheated	24	12.71	7.5	2.163	4.00	3.38	....
2. Cream heated to 143° F. for 30 min.	24	12.55	7.5	2.102	3.79	3.22	4.0
3. Cream heated to 160° F. for 5 min.	24	12.67	7.6	2.082	3.58	2.80	14.5
4. Cream heated to 180° F. for 0 min.	24	12.58	7.6	2.000	3.46	2.64	19.0

the higher temperatures there was a more marked reduction in bound water content and the volume of the cream layer was also greatly reduced.

Table 2 shows that the heating of the skimmilk alone and subsequent adding of fresh cream to it to make a normal milk, gives results similar to those obtained when whole milk is heated except that the reduction in the cream layer and bound water content is much greater.

The heating of the cream to high temperatures does not have a marked effect upon the cream layer although the bound water content of the reconstituted milk was noticeably reduced but not as much as when the plasma only was heated. Apparently when the cream is heated there is a reduction in the bound water content of the hydrophilic fat globule membrane of the cream. From these experiments it might be said that the bound water content of milk colloids does not affect greatly the creaming ability of the milk. The greatest reduction in creaming was obtained in the samples containing the greatest reduction in bound water content it is true, but the differences in bound water reduction shown in Tables 1 and 3 do not indicate any relation to creaming ability exhibited in the same samples.

#### THE RELATION OF BOUND WATER CONTENT TO THE SPECIFIC GRAVITY OF MILK

Freshly drawn milk invariably shows an increase in specific gravity on standing. This is commonly referred to as Rechnagel's phenomenon. Rechnagel (7) associated this change with an increase in the hydration of the proteins. He did not study skimmilk. Quevenne (8) also thought that changes in protein hydration were concerned. Toyonaga (9) obtained results that show that a change in specific gravity was due to volume changes when the fat solidified and that the change did not occur in skimmilk. Fleischman and Wiegner (10) obtained the density increase when milk was held at 15° C. and confirmed the results of Toyonaga. Fleischman (11) observed that the changes of volume of milk with temperature changes are greater than for water. These changes with temperature are attributed principally to variation in hydration of the proteins.

Davies (3) attributes the increase in density on standing to: (1) changes in the specific gravity of the fat due to cooling and partial solidification; (2) hydration of the proteins; and (3) loss of carbon dioxide. Sharp and Hart (12) state that previous temperature history of the milk influences its specific gravity at 15° C. and that this variation is due to the physical state of the fat.

Rechnagel (7) found that the rise in density is regular, and is more rapid at low than at high temperatures and amounts to 0.001. Richmond (13) found the average change to be 0.0006 with a variation from 0.0003–0.0015 and attributes this largely to the increase in density of the fat on solidification. He further states that the effect does not vary seasonally

but may vary with milk from individual cows or from one sample to another.

Freshly drawn mixed milk was used to note if the change in specific gravity was accompanied by a change in bound water content on standing. The milk was quickly cooled in ice water to 40° F. after processing and several experiments were conducted. The specific gravity was determined with the Westphal balance at 15° C. The milk was divided into four lots and comparisons were made of the following: (1) raw milk, freshly drawn; (2) milk heated to 143° F. for 30 minutes; (3) raw skimmilk; and (4) skimmilk heated to 143° F. for 30 minutes. To eliminate dissolved gases as a factor, lots 2 and 4 were heated, and to eliminate butter fat as a factor, experiments with skimmilk were conducted. Determinations were made on the freshly prepared samples, after holding 8 and 24 hours at 40° F. (Table 4.)

TABLE 4

*The relation of bound water content to the specific gravity of milk*

Sample	Hours aged at 40° F.	Per cent solids	Viscosity centipoises	Specific gravity (15° C.)	Per cent bound water
1. Raw milk	0	13.25	2.245	1.0314	2.79
	8	13.25	2.347	1.0328	2.98
	24	13.25	2.387	1.0332	3.18
2. Pasteurized milk	0	13.61	2.183	1.0323	2.48
	8	13.61	2.285	1.0338	2.71
	24	13.61	2.326	1.0340	2.71
3. Raw skimmilk	0	9.44	1.775	1.0359	1.81
	8	9.44	1.836	1.0366	2.05
	24	9.44	1.877	1.0371	2.13
4. Pasteurized skimmilk	0	9.48	1.591	1.0358	1.41
	8	9.48	1.632	1.0372	1.69
	24	9.48	1.694	1.0375	1.81

It will be noted in Table 4 that milk and skimmilk, whether raw or pasteurized, increase in specific gravity and bound water content upon standing at 40° F. The specific gravity of raw milk increased 0.0018, and in the pasteurized milk it increased 0.0017 in 24 hours. When the raw milk was pasteurized the specific gravity increased 0.0009, showing that the dissolved gases or an increase in concentration of solids may play a part in this phenomenon. The difference in specific gravity between the raw and pasteurized skimmilk samples was only 0.0001 when determinations were made on freshly prepared samples.

Results obtained indicate that there is an increase in specific gravity of raw and pasteurized skimmilk on standing which cannot be attributed to either the dissolved gases, or to the solidification of the fat globules since in the skimmilk very little fat is present. The increase was 0.0012 in the raw skimmilk and 0.0017 in the pasteurized skimmilk. The bound water studies indicate that the increase in specific gravity might be attributed to the hydration of the lyophilic colloids.

THE EFFECT OF AGE AND TREATMENT ON THE BOUND WATER CONTENT  
OF VACUUM DRUM DRY SKIMMILK

To note whether there were any changes in the bound water content of vacuum drum dry skimmilk on aging, samples were stored in open and closed cardboard containers. To determine the bound water content, reconstituted mixtures were made that contained approximately 24 per cent total solids. The bound water content was determined on these mixtures made from fresh dry skimmilk, dry skimmilk stored four weeks and dry skimmilk stored eight weeks at room temperature. The results of these trials are recorded in Table 5.

TABLE 5

*The effect of age and treatment on the bound water content  
of vacuum drum dry skimmilk*

Sample*	Hours aged at 40° F.	Per cent solids	Viscosity centipoises	Alcohol No.	Per cent bound water	Grams bound water per gram of solids
1	0	24.09	12.641	5.6	11.22	0.465
	24	24.09	13.530	5.4	14.72	0.611
2	0	24.52	11.160	5.4	9.26	0.377
	24	24.52	12.345	5.4	9.89	0.403
3	0	23.25	10.883	5.4	8.77	0.377
	24	23.25	11.476	5.4	8.81	0.378
4	0	24.95	8.770	5.5	9.30	0.372
	24	24.95	9.560	5.5	10.01	0.401
5	0	24.71	8.533	5.5	7.87	0.318
	24	24.71	9.402	5.5	8.87	0.358

\*1. Reconstituted dry skimmilk—fresh.

2. “ “ “ —four weeks old in closed container.

3. “ “ “ —“ “ “ open “

4. “ “ “ —eight “ “ “ closed “

5. “ “ “ —“ “ “ “ open “

It is evident that there is a decrease in the amount of bound water per gram of total solids as the dry skimmilk becomes older. The decrease in bound water content is more marked in the samples of dry skimmilk stored in open containers. No difference in solubility of the dry skimmilk was noticed when these samples were reconstituted. A decrease of viscosity also occurred on storage of the dry skimmilk. The protein stability was not markedly altered by the periods of storage.

THE EFFECT OF “SUPERHEATING” ON THE BOUND WATER CONTENT  
OF CONDENSED SKIMMILK

The thickening produced in superheated condensed skimmilk is associated with the coagulation of the calcium caseinate. To observe the effect of superheating on the bound water content, a freshly prepared sample of condensed skimmilk was obtained directly from the vacuum pan. Part of the sample was used as the control and the other part was thickened by heating

to 180° F. for 20 minutes in a water bath. The results obtained may be observed in Table 6.

TABLE 6

*The effect of superheating on the bound water content of condensed skim milk*

Sample	Hours aged at 40° F.	Per cent solids	Viscosity centipoises	Per cent acid	Alcohol No.	Per cent bound water	Grams bound water per gram of solids
1. Condensed skim-milk	0	26.27	6.459	0.58	5.0	8.09	0.307
	24	26.27	7.644	0.60	5.3	10.97	0.417
2. Superheated condensed skim-milk	0	26.43	21.124	0.575	5.7	8.99	0.340
	24	26.43	23.525	0.595	5.8	10.57	0.399

Although a large increase in viscosity is characteristic of superheating, there was little change in the bound water content because of the superheating. Both samples had been previously forewarmed in the hot well at 180° F. Superheating produced a noticeable increase in the stability of the proteins as recorded by the alcohol number. The change in viscosity obtained on superheating is not due to hydration but to coagulation.

#### THE EFFECT OF THE INITIAL AGING TEMPERATURE ON THE BOUND WATER CONTENT OF CONCENTRATED MILK PLASMA

The initial aging temperature is known to change some of the properties of dairy products, notably the viscosity of the system. To note if there were any bound water changes, concentrated milk plasma was aged in water baths at 90° F., 70° F., 50° F. and 34° F. and the bound water content then determined.

The results show that more water is bound initially at the higher aging temperature than at the lower temperatures. Therefore the process of imbibition is slower at the lower temperatures.

From these findings, it would seem reasonable to believe that short aging periods at relatively high temperatures would give ice cream mixes characteristics similar to low temperature mixes aged for longer periods. Mueller and Frandsen (14) have shown that about one-fourth of the gelatin content of ice cream mixes could be saved by using an aging temperature of 68° F. for 2 to 4 hours instead of cooling to a low temperature immediately after pasteurization and aging for a longer period as is the custom in ice cream plants.

It was also found by these writers that such ice cream mixes whipped as well as mixes aged at lower temperatures. The results in Table 7 indicate that more water is initially bound at 90° F. than at lower temperatures and this may be responsible for results noted by Mueller and Frandsen.

TABLE 7

*The effect of the initial aging temperature on the bound water content of concentrated milk plasma*

Sample	Hours aged	Per cent solids	Viscosity centipoises	Per cent acid	Alcohol No.	Per cent bound water	Grams bound water per gram of solids
1. Aged at 90° F.	4	13.71	4.859	0.28	8.4	5.52	0.403
	24						
2. Aged at 70° F.	4	13.71	4.701	0.265	8.4	4.58	0.334
	24	13.71	4.859	0.260	8.4	5.38	0.392
3. Aged at 50° F.	4	13.71	4.563	0.265	8.3	4.21	0.307
	24	13.71	4.997	0.280	8.3	5.77	0.421
4. Aged at 34° F.	4	13.71	4.405	0.260	8.2	3.92	0.286
	24	13.71	4.859	0.280	8.1	5.11	0.373

THE EFFECT OF AGING AT LOW TEMPERATURES ON THE BOUND WATER  
CONTENT OF FLUID MILK PRODUCTS

In studying the effect of aging on the bound water content, samples were stored at 40° F. for 4 and 24 hours, after which time the determinations were made in duplicate. Approximately 40 minutes elapsed from the time the samples were obtained until the determinations were made on the fresh samples. Determinations were made again after aging for 4 and 24 hours. In the tables previously presented, the effect of aging at 40° F. is plainly seen. In all cases (Tables 4, 5, 6, 7) the samples aged at 40° F. contained more bound water than the fresh sample. Aging of the ice cream mix 12 to 24 hours has been common practice in the ice cream industry. This is done to enhance the whipping ability of the mix in the freezer. Work by Dahle, Keith and McCullough (15), Hening (16) and others have shown that approximately four hours of aging produces whipping results as satisfactory as 24 hours of aging. Viscosity studies show that much of the increase in viscosity takes place in that four-hour period.

That whipping ability might go hand in hand with bound water content is indicated in Table 8. In this table is shown also that in the mixes which were homogenized at various pressures the viscosity increase during aging is greatest in the four-hour period. The bound water content shows the greatest increase in the first four hours of aging. The increase is slight after that period up to 24 hours, and it is apparent (15) that aging for the purpose of increasing bound water content need not be practiced longer than four hours at 40° F.

Sugar and gelatin were purposely omitted from these mixes because of the interference offered in making the bound water determinations.

Homogenization with single pressures reduced the bound water content, but the bound water content was restored and even slightly increased by dual homogenization as noted in mix sample No. 4. In this case fat clump-

TABLE 8

*Relationship between age of an ice cream mix, viscosity and bound water (mixes contained 14.75 per cent fat and 12.15 per cent M.S.N.F.\*)*

Mix No.	Pressure used	Hours aged at 40° F.	Viscosity centipoises	Per cent bound water
1	0	0	7.9	5.14
		4.0	9.12	5.56
		24.0	8.8	5.29
2	1500	0	12.9	2.43
		4.0	14.1	3.22
		24.0	14.7	3.79
3	3000	0	45.3	1.96
		4.0	52.4	2.23
		24.0	53.23	3.47
4	3000 and 700	0	10.5	5.65
		4.0	12.8	5.93
		24.0	12.4	6.18

\* No gelatin or sugar was used in mix.

ing was destroyed while in the other homogenized samples much clumping took place.

Cream and other products show an increase in bound water content on aging. Previous work (1, 2) shows that fresh raw cream increased about 16 per cent in bound water on aging, while pasteurized cream increased approximately 30 per cent. Heavy cream increases more in bound water on aging than does light cream. The substances associated with the fat also increase in bound water content on aging. This shows that they are partially responsible for the increase in the bound water content of dairy products containing butterfat.

#### THE RELATION OF BOUND WATER CONTENT TO THE VISCOSITY OF SWEET CREAM

Cream was prepared according to the Hening and Dahlberg method (17) and its viscosity and bound water content compared with raw cream and ordinary pasteurized cream. The method used is as follows: Raw cream is pasteurized at 143° F. for 30 minutes and then cooled to 40° F. or lower. It is then warmed to 84° F. in seven minutes and cooled to 48° F. in 14 minutes. The cream is cooled to 40° F. in a refrigerator.

It was thought that since this method produces such a noticeable increase in viscosity, there may be a corresponding increase in bound water content. Therefore, a number of studies were made on fresh cream and cream after 24 hours of storage at 40° F. (Table 9).

The Hening and Dahlberg method of increasing the viscosity of sweet cream shows an increase in the bound water content over ordinary pasteurized sweet cream held at 40° F. for 24 hours, although less bound water is present than is present in aged raw cream.

Hening and Dahlberg found that the increased viscosity obtained by this

TABLE 9

*The effect of temperature treatment on the bound water content and viscosity of sweet cream*

Treatment	Hours aged at 40° F.	Per cent solids	Viscosity centipoises	Alcohol No.	Per cent bound water	Grams bound water per gram solids
1. Unheated	0	40.25	24.691	5.0	5.39	0.134
	24	40.25	29.796	5.2	6.26	0.156
2. Heated to 143° F. for 30 min.	0	41.40	22.653	5.2	3.94	0.095
	24	41.40	25.918	5.3	5.11	0.123
3. Special temperature treatment	0	41.49	20.000	5.2	4.95	0.119
	24	41.49	28.751	5.4	5.64	0.136

method of treating cream could not be associated with increased clumping of the fat globules. These results were substantiated by the writers. From the data obtained it is shown that the hydrophylic colloids become more hydrated by this temperature treatment, though this is not necessarily offered as an explanation for the viscosity change obtained.

#### THE RELATION OF BOUND WATER CONTENT TO VISCOSITY

In this work a study was made of the data presented to show, if possible, the relationship between viscosity and bound water content in dairy products. In viscosity studies we are usually dealing with lyophilic systems, since in general the lyophobic sols exhibit a viscosity which approaches very closely the viscosity of the pure dispersions medium, and which increases only slightly with increasing concentration of dispersed material. In dairy products we are dealing with a heterogeneous system. Nevertheless, changes in bound water content should produce changes in viscosity although the differences may not be well defined.

The viscosity studies in Tables 4, 6, 7, 8 and 9 show that the aging of liquid dairy products at low temperatures usually causes an increase in viscosity and bound water content. It appears in these tables that the greater the amount of solids present, the greater is the increase in viscosity on aging. In many of these instances the increases do not appear very great but when the initial viscosities are taken into consideration they are significant. Undoubtedly the increase in viscosity on aging is due to the increase in bound water content of the lyophilic colloids. These results agree with those of Evenson and Ferris (18) and Dahlberg and Hening (19) who show that aging increases the viscosity of milk and cream.

The pasteurization of milk and cream causes a decrease in the viscosity and the bound water content. The viscosity of milk and cream did not return to the original reading even after aging at low temperatures as can be seen in Tables 1, 2, 3, 4 and 9. Many workers, especially Dahlberg and Hening (19), Evenson and Ferris (18), and Babcock and Russell (20) have

demonstrated that heating of whole milk causes a diminution of the viscosity.

Table 8 shows that homogenization increased the viscosity of ice cream mixes and decreased the bound water content. The greater the pressure of homogenization the more pronounced was the increase in viscosity and decrease in bound water content. It will be noted in mix sample No. 4 of Table 8 that dual homogenization decreased the viscosity and increased the bound water content. Undoubtedly the increase in viscosity in single homogenization is due to the clumping of the fat globules, and to greater fat surface.

#### THE RELATION OF BOUND WATER TO PROTEIN STABILITY

The alcohol stability determination is probably the most accurate test used to measure the stability of the proteins in certain dairy products. A relatively high alcohol number is usually associated with greater heat and acid stability, and stability to homogenization and freezing.

Throughout this investigation, where feasible, the alcohol number was determined to note if any correlation between alcohol stability and bound water content was obtained. Some of the more positive results are recorded in Table 10.

TABLE 10

*The relation of bound water content to protein stability in some dairy products*

Sample	Hours aged at 40° F.	Per cent solids	Alcohol No.	Per cent bound water
1. Raw milk	24 *	11.92	8.1	3.75
2. Milk pasteurized at 143° F. for 30 min.	24	12.09	9.1	3.56
3. Milk pasteurized at 160° F. for 5 min.	24	11.94	9.3	3.06
4. Milk pasteurized at 180° F. momentarily	24	12.11	9.3	3.30
5. Raw cream	0	40.25	5.0	5.39
	24	40.25	5.2	6.26
6. Cream pasteurized at 143° F. for 30 min.	0	41.40	5.2	3.94
	24	41.40	5.3	5.11
7. Condensed skim milk	0	26.27	5.0	8.09
	24	26.27	5.3	10.97
8. Superheated condensed skim-milk	0	26.43	5.7	8.99
	24	26.43	5.8	10.57

The aging of dairy products at low temperatures for 24 hours does not have any marked effect upon the alcohol stability, although the trend throughout this work (1, 2) does in some cases show a slight increase in alcohol stability with the increase in bound water content. In most cases the alcohol stability determination either showed that a slight increase or no change in stability resulted upon aging the sample.

That high heat treatment usually results in a decrease in bound water content and an increase in alcohol stability is shown in Tables 2, 3 and 10.

It was found by the writers in a previous investigation (2) that homogenization decreased the bound water content of milk plasma mixes containing butterfat and also decreased the alcohol stability. As the pressure is increased there is an increase in the degree of fat clumping and a decrease in the alcohol stability and bound water content. Dual homogenization decreased the fat clumping, increased the bound water content, and also increased the alcohol stability of the mix.

The results noted in Table 10 indicate that some dairy products, notably cream and condensed skimmilk, do increase in alcohol stability and bound water content on aging.

Previous results (2) also show that milk stabilizing salts slightly increased the alcohol stability and bound water content, while the milk destabilizing salts decreased the alcohol stability and the bound water content.

#### SUMMARY AND CONCLUSIONS

The bound water content of the milk colloids does not appear to contribute to the creaming ability of milk. High temperature treatment of the whole milk or skimmilk is detrimental to the creaming ability and bound water content of the milk while high temperature treatment of the cream portion does not affect creaming to any great extent.

The increase of the specific gravity of fresh milk and skimmilk on aging (Rechnagel's phenomenon) is thought to be partially due to the increase in the bound water content of the proteins and other hydrophilic substances present in milk.

Vacuum roller dry skimmilk loses some of its bound water with age. The greatest amount is lost during the first few weeks of storage. The dry skimmilk stored in open containers was affected more than the dry skimmilk stored in air-tight containers.

Superheating of condensed skimmilk had no appreciable effect upon the bound water content.

Short aging periods of concentrated milk plasma at relatively high temperatures resulted in only slightly more bound water than longer aging periods at low temperatures.

Invariably aging caused an increase in bound water content, regardless of the treatment given the sample.

The Dahlberg and Hening method of increasing the viscosity of cream by temperature treatment resulted in an increase in the bound water content of the cream.

Pasteurization of milk or cream lowered viscosity and bound water content.

Homogenization of mixes with one pressure increased viscosity and decreased the bound water content, while dual homogenization of the same product showed an increase in bound water content and viscosity.

The protein stability as measured by the alcohol number usually showed little change with changes in bound water content unless large changes were noted in the amount of bound water present. However, heating of milk to high temperatures decreased the bound water content but increased the stability toward alcohol, while superheating of condensed skim milk resulted in increased stability with practically no change in bound water content.

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# THE RELATION BETWEEN ACID DEFECTS AND HYDROGEN ION CONCENTRATION IN BRICK CHEESE

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Measurements of hydrogen ion concentration have proved to be a useful means of following acid development in American (1, 6, 7, 8) and Swiss cheese (2, 3, 4, 5) during manufacturing and the early part of curing. These measurements in American cheese are so closely related to the flavor and body characteristics of the ripened cheese that they can be used advantageously in the control of acid. Observations are reported in this discussion which seem to indicate that measurements of hydrogen ion concentration of Brick cheese during the first few days of curing may be a very useful guide for controlling subsequent manufacturing processes.

The successful making of Brick cheese with *Streptococcus lactis* starter requires the development of acidities which, if they are not carefully controlled, may cause defective cheese. The flavor, body and texture of Brick cheese are all directly influenced by the acidity development. Excessive amounts of acid delay ripening, cause undesirable acid or sour flavors, and crumbly, mealy bodied cheese. Low acidity, on the other hand, encourages the development of abnormal, gassy fermentations and causes excessively open texture and abnormal flavors in the cheese. Increases in the yield are usually attempted by reducing the acidity so that more moisture will be retained in the cheese. Such manipulations may produce very sweet or, what is more surprising, very acid cheese.

It is not always easy to detect abnormally acid or sweet cheese by sense of taste or body characteristics soon enough in the curing process to prevent repetition of the fault. Measurements of hydrogen ion concentration were, therefore, made during the first week of curing 72 lots of cheese and these values have been correlated with the criticisms of competent judges. The results are reported in this paper.

Acid measurements were made with the quinhydrone electrode, saturated calomel half-cell and a Leeds-Northrup portable potentiometer. Measurements are reported in pH units. Samples of cheese were taken by cutting each loaf in half across the long dimension and then removing a cross section slice. The whole slice, excluding the rind, was used for the analyses.

## DAILY VARIATIONS IN ACIDITY

Daily variations in acidities of 7 typical lots of Brick cheese are shown in Figure 1. Acid measurements were made on these lots when they were taken

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from the hoops just before salting, again on the third day just after salting, and daily, thereafter, until the 7th day after making. The last measurement was made at the time of paraffining on about the 14th day. All 7 lots varied somewhat in acid during this period, the maximum variation equaling 0.15 pH units. The pH on the third day after making approximated the minimum for most of the lots. Toward the end of the week the acidities generally approximated the values observed on the third day. During the second week the acid values of these seven lots became relatively divergent. Such variations must be expected because of sampling different loaves of cheese, changing salt concentrations, bacteriological activity and the limits of accuracy of the method of acid measurement.

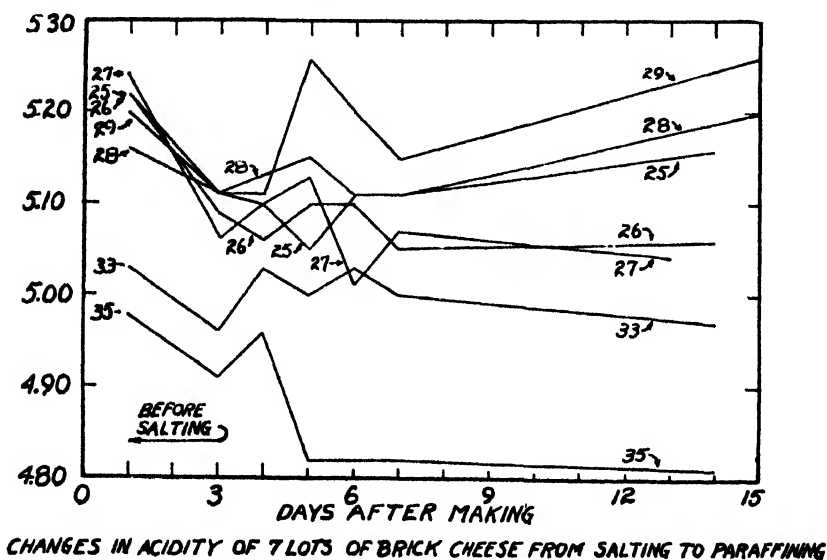


FIG. 1

After the first week of curing there was in general, a slight increase in the average pH of all lots. This trend continued as the cheese ripened. Individual lots acted differently in rate of pH increase. Such variations explain why, during the later stages of ripening, individual lots of sweet and acid Brick cheese may actually have the same pH values. This has also been observed in ripening American cheese.

#### RELATION BETWEEN ACIDITY OF CHEESE AND ACID DEFECTS

Forty-eight lots of Brick cheese have been classified in Table 1 according to the "Acid Grade." The acid grade indicates the degree of acidity in flavor and body detected by the judges. Satisfactory cheese is called "sweet" and given an acid grade of zero. "Trace of acid," "acid," "very acid" and "sour" indicate increasing degrees of acidity and are given grades of 1, 2,

3 and 4, respectively. The average pH values calculated for the cheese in each acid grade tended to decrease with the exaggeration of the defect. Sweet cheese had an average pH of 5.09 three days after making while sour cheese had an average pH of 4.92. Comparison of average values on the third and seventh days shows no significant differences. Actually the averages of the pH of all the cheese in the four acid grades were identical on these two days.

TABLE 1  
*Average pH of Brick cheese in each acid grade*

Acid grade	Number of lots	Average acidity	
		3 days after making	7 days after making
		<i>pH</i>	<i>pH</i>
Sweet	28	5.09	5.12
Trace of acid	4	5.03	5.02
Acid	8	4.95	4.95
Very acid	3	4.99	5.03
Sour	5	4.92	4.91

The relation between acid measurements on the third and seventh days after making and the acid grade is shown in Figure 2. Numerical values representing the average acid grade for corresponding pH classes have been averaged, then plotted and a smooth curve has been drawn to fit the data obtained on the third day. Data from the seventh day were too scattered in the high acid range to justify a smooth curve. This figure, as well as Table 1, indicates that pH measurements as a whole were directly related to the flavor and body characteristics of the cheese. As the pH decreased, the cheese became increasingly acid in the opinion of the judges. Third day measurements, however, seem to be more closely related to the acid grade than those

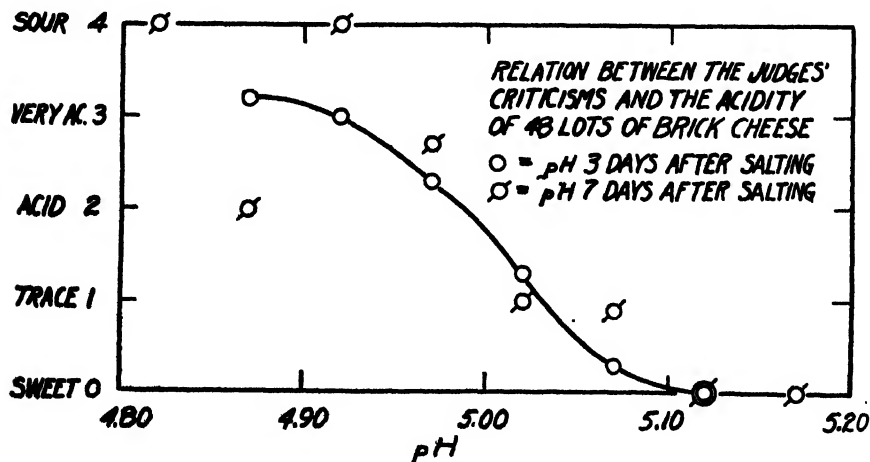


FIG. 2

obtained on the seventh day. Measurements on the fourth day after making approximate the significance of those on the third day.

Any degree of acid defect may be regarded as undesirable. All forty-eight lots of cheese were therefore divided into sweet and acid defective groups. The lots of cheese in each group were classified according to their acidity on the third day after making and the results are shown in Table 2.

TABLE 2

*Relation between the judges' criticisms and the acidity of Brick cheese 3 days after making*

Acidity of cheese 3 days after making	Total of sweet and acid lots	Sweet cheese	
		Number	Per cent
<i>pH</i>	<i>Number</i>		
5.10 to 5.19	14	14	100
5.00 to 5.09	21	13	62
4.90 to 4.99	9	1	11
4.80 to 4.89	4	0	0

When the pH of the cheese was 5.00 to 5.09 a slight majority of lots were sweet but there were almost as many acid defective cheese in this grade. Below pH 5.00 only one lot was called sweet. Table 3 illustrates the same arrangement of data obtained from measurements of pH on the seventh day. Here again pH values of 5.10 or more are associated only with sweet cheese, while below this value some cheese are sweet but more are acid defective. It seems safe to state that pH values of Brick cheese should never fall below 5.10 on the third day or seventh day after making if acid defective cheese is to be avoided.

TABLE 3

*Relation between the judges' criticisms and the acidity of Brick cheese 7 days after making*

Acidity of cheese 7 days after making	Total of sweet and acid lots	Sweet cheese	
		Number	Per cent
<i>pH</i>	<i>Number</i>		
5.20 to 5.29	3	3	100
5.10 to 5.19	17	17	100
5.00 to 5.09	16	8	50
4.90 to 4.99	7	0	0
4.80 to 4.89	5	0	0

Manufacturers or buyers may have difficulty in always measuring pH on the third day after making. Values of pH on other days as indicated in Figure 1 may vary widely from those made on the third day. However, even random measurements during the first seven days after making still have some value as indicated in Table 4. Here, pH measurements made on 72 lots of Brick cheese during the first week of curing have been classified in the same manner as the data in Tables 2 and 3. The highest or most favorable pH value observed for each lot during the first seven days after making was

selected as the basis of the acid classification in Table 4. Even when these values are used to predict the acid grade of the cheese the pH 5.10 standard still maintains its significance. The chances of making acid defective cheese are very slight when the pH during the first week of curing remains at 5.10 or more.

TABLE 4

*Relation between pH and quality when cheese is classified by the highest pH values observed during the first week after making*

Highest pH observed during first week	Total of sweet and acid lots	Sweet cheese	
pH	Number	Number	Per cent
5.20 to 5.29	4	4	100
5.10 to 5.19	27	26	93
5.00 to 5.09	21	7	35
4.90 to 4.99	15	1	6
4.80 to 4.89	5	0	0

Cheese buyers who receive Brick cheese after approximately two weeks of curing cannot apply so well the pH method of judging acidity. At two weeks of age the pH values of some lots of acid cheese may actually be well above the 5.10 standard of the first week of curing. This fact is illustrated in Table

TABLE 5

*Relation between the judges' criticisms and the acidity of Brick cheese at paraffining*

Acidity of cheese at paraffining*	Total of sweet and acid lots	Sweet cheese	
pH	Number	Number	Per cent
5.20 to 5.29	14	12	86
5.10 to 5.19	19	15	79
5.00 to 5.09	25	9	36
4.90 to 4.99	11	2	18
4.80 to 4.89	3	0	0

\* Paraffining occurred 13 to 18 days after making.

5 which shows two acid lots of cheese with a pH of more than 5.20 and approximately two-thirds of all acid lots under observation above pH 5.00. Practically all pH values for sweet cheese fall in the same classes with about one-third in the pH 5.20 to 5.29 group. This over-lapping of the acidities of sweet and acid cheese at this time makes measurements of little value unless they happen to be extremely high or low. Actually, however, pH measurements are not necessary at this time, since by the fourteenth day after making, acid flavor and short body are easily detected by any competent judge.

#### CONCLUSIONS

The pH of Brick cheese three days after making is a useful index of the acid characteristics of the ripened cheese. The minimum pH should not be

less than 5.1 at this time. The fact that the observed limit of acidity approximates that desired in American cheese indicates the similarity of the two types of cheese and emphasizes the significance of pH in controlling the elastic properties and the flavor of fresh cheese. Undoubtedly characteristic acidity limits distinguish other varieties of cheese.

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# DETECTING THE NEUTRALIZATION OF MILK WITH THE CRYOSCOPE

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In the examination of milk for the addition of neutralizers, it is frequently impossible to say definitely that a sample has been adulterated. This is probably due to the fact that the present tests which have been proposed are based on a characteristic or constituent of milk that is too variable.

Tillmans and Luckenback (11), for example, suggest a method (later modified by Sommer (9)), which is based on the difference between the buffer capacity of normal and neutralized milk of the same acidity.

Mojonnier (7) devised a method for the determination of lime in dairy products. If the amount of lime found in a suspected sample exceeds the normal amount, the sample may be considered to be neutralized.

The pH (6) may be determined either with indicators, such as bromthymol blue, or by the electrometric method; and neutralization may be deduced from an abnormally alkaline pH.

A method was proposed by Nottbohm (8) for determining the sodium-potassium ratio in the suspected milk. An abnormally greater proportion of sodium to potassium indicated neutralization.

Since the present methods are not generally accepted, it was thought that a method, to be acceptable, should be based upon some constant characteristic of milk such as the freezing point. A method such as this was published by Koenig and Kluge (5). Although they reported the results on only three neutralized samples of milk, the method appeared to be of value. They stated that if the corrected freezing point lies below  $- .554^{\circ} \text{C.}$ , neutralization is indicated. The corrected freezing point is obtained in the following manner: Subtract 7 from the observed Soxhlet-Henkel degrees acidity and multiply the result by .007. Subtract this figure from the observed freezing point.

In studying neutralized milk, the greatest difficulty involved is the wide variation of the original acidity of different samples of milk. Caulfield and Riddell (2) report variations from .098 to .295 per cent acidity of milk from cows in Kansas. Experience in this laboratory is in agreement with Caulfield and Riddell; a range of .09 to .245 per cent acidity was found in 4,000 samples of milk. Consequently, if an unknown sample of milk has an acidity of from .09 to .25 per cent it cannot be definitely regarded as sour, normal, or neutralized by the determination of acidity alone.

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Hortvet (3), Keister (4), Bailey (1), and others have done a considerable amount of work on the freezing point and its relation to the souring of milk. There are also many reports regarding the effect of heat, garget, colostrum, skimming, age of cow, breed of cow, period of lactation, season of the year, etc., all of which show that these factors have no appreciable effect upon the freezing point of pure milk. There was yet to be shown, therefore, the normal variations that might be expected between the developed acidity of normal milk and its freezing point. It was also necessary to show the effect of various neutralizers on the freezing point.

#### EXPERIMENTAL

All of the work herein reported was done on samples of known purity from herds of two to twenty cows each. Each sample was examined for bacteria count, acidity, pH, and freezing point. It was then allowed to sour spontaneously and again examined for acidity, pH, and freezing point. The sour sample was then divided into two to eight parts depending upon the nature of the experiment and neutralized with NaOH,  $\text{NaHCO}_3$ ,  $\text{Na}_2\text{CO}_3$ , and  $\text{MgCO}_3$  in the dry form. These samples were in turn examined for acidity, pH, and freezing point. The relationship existing between pH and freezing point is not as definite as the one between acidity and freezing point. For this reason and for the sake of brevity, all the pH readings obtained are not reported. However, they were run on all samples as a check on the acidity.

Acidity was determined by pipetting 17.6 ml. of the sample into a white container, adding  $\frac{1}{2}$  ml. of 1 per cent phenolphthalein and titrating to a faint pink color with .1 N NaOH. The number of milliliters divided by 20 equals the per cent acidity calculated as lactic acid and was so recorded.

Bacteria counts were made on the fresh samples by the Breed direct method. In no case did the count exceed 300,000 and averaged 100,000 for all samples examined. The samples were therefore considered to be devoid of developed acidity.

Freezing points were determined with a Hortvet (3) cryoscope, the procedure given in Standard Methods of Milk Analysis (10) being followed exactly. A blower of the type used in electric insect spray guns was employed as a source of compressed air. An adjustable type vacuum windshield wiper, the arm of which was hooked to the stirrer, served as a means of mechanically agitating the milk during freezing.

The determinations of pH were made electrometrically with a quinhydrone electrode.

#### RESULTS

Twenty-eight samples of raw milk were allowed to sour spontaneously and freezing points determined at various acidities. These results are given in Table 1. If each sample is plotted, it can be shown that there is a

TABLE 1  
*Effect of souring on freezing point*

Sample number	Acidity	Freezing point - °C.	Sample number	Acidity	Freezing point - °C.
2	.145	.540	13	.12	.548
	.18	.568		.195	.570
5554	.145	.557	224	.14	.557
	.235	.594		.175	.567
682	.22	.550	899	.155	.548
	.32	.582		.19	.558
3485	.11	.545	4018	.15	.547
	.13	.547		.22	.575
				.32	.609
4569	.15	.552		.36	.637
	.205	.562		.40	.645
	.24	.575		.50	.677
	.44	.632		.60	.716
	.56	.660			
	.61	.697	113	.135	.550
				.18	.562
21	.15	.548		.24	.588
	.20	.566		.39	.638
	.24	.600		.50	.697
	.47	.680			
	.59	.719	22	.15	.548
				.24	.600
131	.13	.550			
	.24	.588	5290	.155	.540
2380	.14	.542		.30	.590
	.30	.609	1854	.15	.558
				.21	.580
36	.135	.558			
	.23	.578	4019	.145	.541
	.35	.620		.24	.580
	.37	.622		.33	.611
	.53	.675		.45	.645
				.61	.698
4408	.155	.551			
	.215	.580	3900	.11	.548
	.295	.601		.225	.584
	.41	.631		.30	.609
	.53	.674		.455	.640
				.605	.697
1333	.15	.540	1310	.18	.553
	.23	.562		.215	.568
	.295	.592			
	.375	.632	371	.155	.533
	.52	.657		.295	.576
			6137	.165	.542
				.215	.562
370	.165	.540			
	.215	.560	5290	.15	.541
	.33	.599		.235	.572
	.46	.632			
	.57	.674	5289	.16	.540
				.24	.591
5328	.16	.550			
	.20	.590			

straight line relationship between developed acidity and freezing point. This relationship varies from sample to sample probably due to (a) differences in the original titratable acidity of the milk and (b) differences in the amount of lactose and any other fermentable substances present. The extremes found in all samples studied—including those of Keister's (4) and Bailey's (1) are shown in Figure 1.

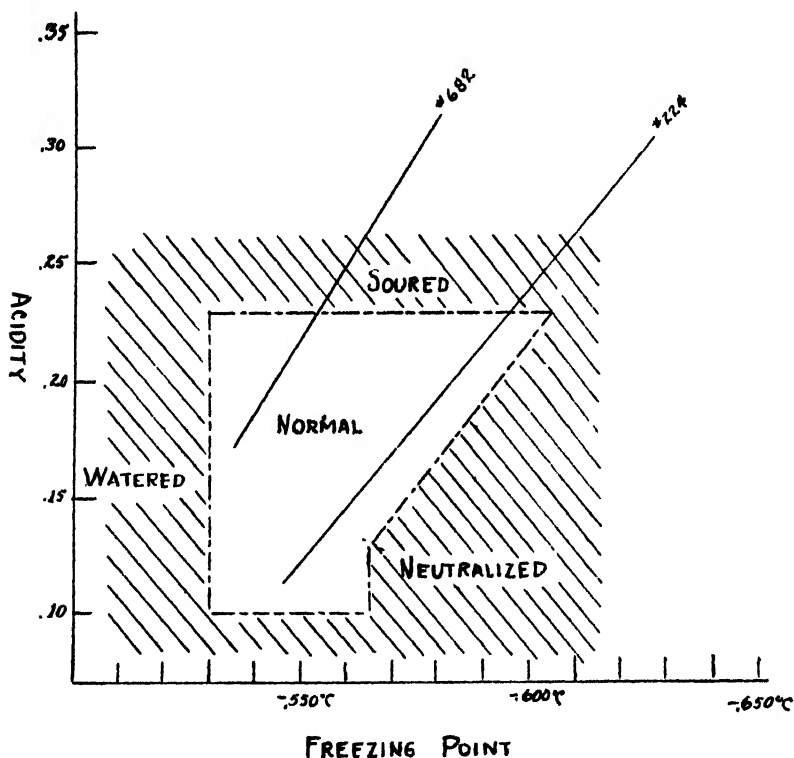


FIG. 1. Maximum (#224) and minimum (#682) freezing-point-acidity curves found in 40 samples of normal milk including those of Bailey's and Keister's. The shaded area represents abnormal milk.

It was recognized that only a small number of samples were tested and those from just one section of the country. A certain leniency, therefore, was allowed when determining whether or not a sample was neutralized. The shaded portion of Figure 1 was considered abnormal for pure milk and any point in this area was judged watered, neutralized, or soured according to its location.

In Table 2 is recorded the effect of the addition of four kinds of neutralizers on the freezing point and acidity. In columns eight and nine an attempt was made to characterize the sample as if it had been of unknown origin. This was based on the acidity and freezing point of the sample

according to Figure 1. If the location of the plotted point was in the shaded area, the sample was considered neutralized; if on the dotted line, doubtful; and if in the clear area, normal. As will be observed from the table, of the 45 samples that were neutralized from .015 to .29 per cent

TABLE 2  
*Effect of neutralization on freezing point*

Sample number	Normal		Soured		Neutralized		Max. fr. pt. allowable for acidity, if normal. - °C.	Characterization of sample, if unknown
	Acid %	Fr. pt. - °C.	Acid %	Fr. pt. - °C.	Acid %	Fr. pt. - °C.		
(neutralized with NaHCO <sub>3</sub> )								
2	.14	-.540	.205	-.568	.12	-.608	-.565	neutralized
13	.12	.548	.195	.570	.125	.608	.565	"
554	.145	.557	.235	.594	.15	.634	.575	"
224	.14	.557	.175	.567	.145	.572	.570	doubtful
682	.22	.550	.32	.582	.22	.607	.600	"
899	.155	.548	.19	.558	.15	.581	.575	neutralized
3485	.11	.545	.13	.547	.11	.560	.565	normal
22	.15	.548	.24	.600	.215	.741	.597	neutralized
131	.13	.550	.24	.588	.12	1.450	.565	"
1310*	.18	.553	.215	.568	.14	.652	.570	"
370*	.165	.540	.215	.560	.145	.800	.575	"
370	.165	.540	.215	.560	.20	.864	.595	"
371*	.155	.533	.295	.576	.115	.995	.565	"
6137*	.165	.542	.215	.562	.15	.646	.575	"
5290*	.15	.541	.235	.572	.16	.650	.577	"
5328*	.16	.550	.255	.585	.19	.651	.590	"
(neutralized with Na <sub>2</sub> CO <sub>3</sub> )								
3900*	.11	.548	.225	.584	.16	.628	.577	"
3900	.11	.548	.225	.584	.12	.615	.565	"
371*	.155	.533	.295	.576	.06	.672	.565	"
6137*	.165	.542	.215	.562	.105	.600	.565	"
5290*	.15	.541	.235	.572	.12	.621	.565	"
5289	.16	.540	.24	.591	.14	.630	.570	"
5328*	.16	.550	.255	.585	.185	.604	.585	"
(neutralized with NaOH)								
5290	.155	.540	.30	.590	.01	.640	.565	"
2388	.14	.542	.30	.609	.08	.628	.565	"
2	.15	.548	.24	.600	.12	.600	.565	"
1854	.15	.558	.21	.580	.18	.596	.585	"
13	.14	.550	.21	.561	.15	.582	.575	doubtful
36*	.135	.558	.23	.578	.12	.580	.565	neutralized
4019	.145	.541	.24	.580	.125	.592	.565	"
4019*	.145	.541	.24	.580	.105	.589	.565	"
4408	.155	.551	.215	.580	.16	.582	.577	doubtful
4408*	.155	.551	.215	.580	.155	.580	.575	"
1310*	.18	.553	.215	.568	.155	.570	.575	normal
371*	.155	.533	.295	.576	.09	.591	.565	neutralized
6137*	.165	.542	.215	.562	.02	.580	.565	"
5290*	.15	.541	.235	.572	.04	.584	.565	"
5328*	.16	.550	.255	.585	.20	.590	.600	normal

TABLE 2.—(Continued)

Sample number	Normal		Soured		Neutralized		Max. fr. pt. allowable for acidity, if normal. — °C.	Characterization of sample, if unknown
	Acid %	Fr. pt. — °C.	Acid %	Fr. pt. — °C.	Acid %	Fr. pt. — °C.		
(neutralized with MgCO <sub>3</sub> )								
36	.135	.558	.23	.578	.12	.578	.565	neutralized
1333	.15	.540	.23	.562	.20	.565	.595	normal
1310*	.18	.553	.215	.568	.17	.595	.582	neutralized
371	.155	.533	.295	.576	.04	.580	.565	"
6137*	.165	.542	.215	.562	.14	.562	.570	normal
5290*	.15	.541	.235	.572	.155	.588	.575	neutralized
5328*	.16	.550	.255	.585	.175	.586	.582	doubtful

\* Neutralized portions heated to 160° F. for 20 seconds.

acidity, 5 would have been considered normal, 4 doubtful, and 36 neutralized.

Since it was necessary to heat the neutralized samples in order to obtain the correct acidity and also to assist the neutralization reaction, a study was made of the effect of heating on neutralized samples of milk. The samples were heated to 160° F. for 20 seconds and cooled immediately. Some of these results are given in Table 3. It was found that heat has a

TABLE 3

*Effect of heat on freezing point of normal, soured, and neutralized samples*

Sample		Normal	Normal heated	Soured	Soured heated	Neutralized	Neut. heated
4019 (NaOH)	acid	.145	.13	.24	.235	.125	.105
	pH	6.60	6.66	6.06	6.15	6.61	6.81
	fr. pt.	.541	.544	.580	.579	.592	.589
4408 (NaOH)	acid	.155	.15	.215	.215	.16	.155
	pH	6.55	6.57	6.20	6.17	6.63	6.58
	fr. pt.	.551	.550	.580	.579	.582	.580
3900 ( $Na_2CO_3$ )	acid	.11	.105	.225	.205	.16	.12
	pH	6.87	6.87	6.03	6.15	6.61	6.83
	fr. pt.	.548	.549	.584	.579	.628	.615
1333 ( $MgCO_3$ )	acid	.15	.14	.23	.21	.20	.14
	pH	6.69	6.72	6.21	6.35	6.46	6.72
	fr. pt.	.540	.538	.562	.559	.565	.560
370 ( $NaHCO_3$ )	acid	.165	.15	.215	.21	.20	.145
	pH	6.61	6.60	6.40	6.37	7.09	7.49
	fr. pt.	.540	.538	.560	.560	.864	.800

variable effect upon the freezing point of a neutralized sample depending upon the kind of neutralizer used. In all cases, the freezing point of the heated neutralized sample was higher than the same sample which was not heated and lower than the soured sample that was not neutralized. This

indicates that once a sample of milk has been neutralized, heating will not affect the accuracy of the cryoscopic method of detection of neutralization.

The effect of different neutralizers on the freezing point of the same sample was next considered. In this study, an attempt was made to neutralize the samples to the same acidity or pH. This was found difficult particularly since all neutralizers were added in the dry form to relatively small amounts of milk (200 ml.). Furthermore, the true acidity could not be determined until after the sample had been heated. It was thought inadvisable to heat the sample more than once since prolonged heating would have a concentrating effect and therefore alter the freezing point. It was found that NaOH and  $MgCO_3$  depressed the freezing point the least,  $Na_2CO_3$  next, and  $NaHCO_3$  the most. Ordinary baking soda ( $NaHCO_3$ ) is probably most frequently used as a neutralizer on the farm, which, fortunately, has been shown to be the easiest to detect. These results are given in Table 4.

TABLE 4  
*Effect of different neutralizers on same sample*  
(Neutralized samples heated)

Sample		Normal	Soured	Neut. NaOH	Neut. $NaHCO_3$	Neut. $Na_2CO_3$	Neut. $MgCO_3$
36	acid	.135	.235	.12			.12
	pH	6.61	6.14	6.76			6.85
	fr. pt.	.558	.578	.580			.579
1310	acid	.18	.215	.155	.14	.14	.17
	pH	6.51	6.29	6.60	7.02	6.70	7.23
	fr. pt.	.553	.568	.570	.652	.600	.595
371	acid	.155	.295	.09	.115	.06	.04
	pH	6.55	5.89	7.01	7.61	7.41	7.60
	fr. pt.	.533	.576	.591	.995	.672	.580
6137	acid	.165	.215	.02	.15	.105	.14
	pH	6.58	6.27	8.25	6.82	7.03	6.73
	fr. pt.	.542	.562	.580	.646	.600	.562
5290	acid	.15	.235	.04	.16	.12	.155
	pH	6.61	6.15	7.80	6.82	6.97	6.63
	fr. pt.	.541	.572	.584	.650	.621	.588
5328	acid	.16	.255	.20	.19	.185	.175
	pH	6.59	6.14	6.56	6.76	6.55	6.59
	fr. pt.	.550	.585	.590	.651	.604	.586

The next question of interest was the effect of further souring on the freezing point of a neutralized sample. As will be seen from a study of Table 5, the straight line relationship of freezing point and acidity is still maintained in the range studied (up to .60 per cent). However, the depression of the freezing point due to neutralization persists. These results are shown graphically in Figure 2, and from them, it may be concluded that

TABLE 5

*Effect of further souring after neutralization on freezing point*

Sample		Normal	Soured	Neut.	Neut. and soured	Neut. and soured
5328 (NaOH)	acid	.16	.255	.20	.26	.41
	pH	6.59	6.14	6.56	6.20	5.58
	fr. pt.	.550	.585	.590	.612	.671
5328 (NaHCO <sub>3</sub> )	acid	.16	.255	.19	.235	.38
	pH	6.59	6.14	6.76	6.48	5.67
	fr. pt.	.550	.585	.651	.665	.725
5328 (Na <sub>2</sub> CO <sub>3</sub> )	acid	.16	.255	.185	.28	.36
	pH	6.59	6.14	6.55	6.03	5.78
	fr. pt.	.550	.585	.604	.640	.670
5328 (MgCO <sub>3</sub> )	acid	.16	.255	.175	.30	.375
	pH	6.59	6.14	6.59	5.94	5.87
	fr. pt.	.550	.585	.586	.637	.670
5289 (Na <sub>2</sub> CO <sub>3</sub> )	acid	.16	.24	.14	.23	
	pH	6.58	6.13	6.93	6.37	
	fr. pt.	.540	.591	.630	.660	
22 (NaHCO <sub>3</sub> )	acid	.15	.24	.215	.30	.46
	pH	6.62	6.01	6.79	6.31	5.83
	fr. pt.	.548	.600	.741	.836	.897
2388 (NaOH)	acid	.14	.30	.08	.33	.42
	pH	6.58	5.97	6.90	5.60	5.42
	fr. pt.	.542	.609	.628	.725	.771
2 (NaOH)	acid	.15	.24	.12	.21	.34
	pH	6.62	6.01	6.58	6.08	5.56
	fr. pt.	.548	.600	.600	.670	.721

further souring does not affect the accuracy of the cryoscopic method for the detection of neutralization.

As a check on the way an inexperienced worker would be able to detect neutralizer, an assistant was given 14 samples of milk of unknown origin. To some of these samples NaHCO<sub>3</sub> had been added in amounts varying from a tablespoon (8 gm.) to  $\frac{1}{2}$  cup (160 gm.) per 10 gallons of milk. He was then asked to judge the milk by acidity and freezing point alone without knowing if the samples had been neutralized or not. His results given in Table 6, indicate that he failed to detect neutralizer in No. 6 (possibly because the original sample may have been watered) and considered the sample containing a tablespoon of baking soda per 10 gallons of milk as doubtful. These results indicate that the test requires no special knowledge other than that of the maximum freezing points allowable for pure milk.

## DISCUSSION

It is recognized that only a few samples of milk have been studied in determining the efficiency of this test. It was felt, however, that the num-

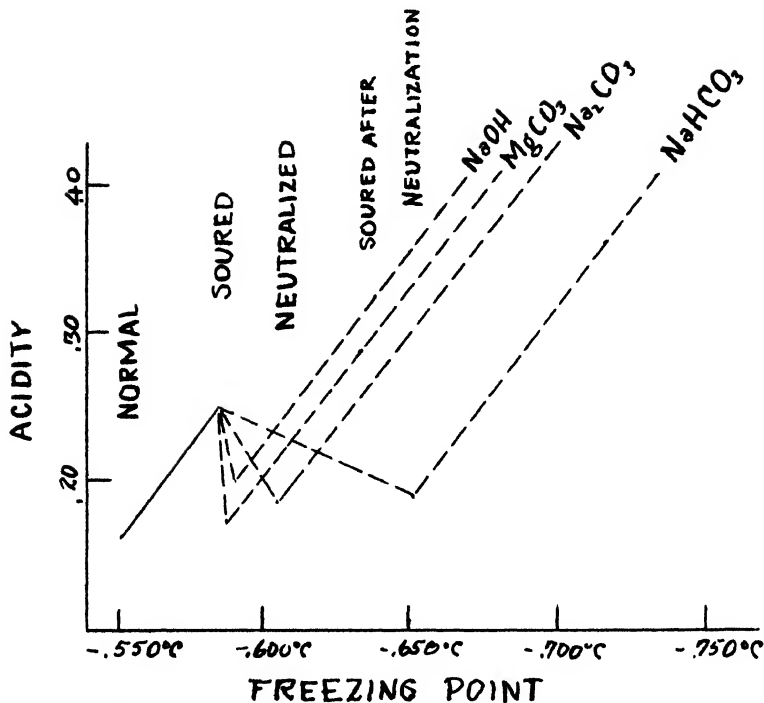


FIG. 2. Effect of further souring after neutralization on the freezing point.

TABLE 6

Results obtained on "prepared unknowns" as analyzed by an inexperienced worker

Sample number	Original sample		Results		
	Acid	Amount neutralizer added to 10 gal. milk	Acid	Fr. pt. - °C.	Remarks
1	.15	none	.155	.482	watered
2	.15	"	.15	.559	normal
3	.18	"	.27	.521	sour—watered
4	.18	"	.455	.628	" —normal
5	.25	80 grams	.23	.650	neutralized
6	.28	"	.25	.540	sour—normal (possibly watered)
7	.20	"	.165	.645	neutralized
8	.265	8 grams	.26	.622	sour—normal (possibly neutralized)
9	.30	160 grams	.245	.754	neutralized
10	.22	40 grams	.19	.643	"
11	.21	"	.20	.624	"
12	.195	"	.18	.609	"
13	.19	80 grams	.165	.611	"
14	.24	"	.235	.647	"

ber was sufficient to warrant its use in this particular section of the country, and it is hoped that work can be done elsewhere which will prove or disprove the value of this test. It has been used in this plant for several months and in the few neutralized samples found by this test, the producer admitted using baking soda. It may be said that this test has definitely increased the efficiency of our field work among the producers.

It is further hoped, that should this test prove satisfactory for milk, it can also be applied to cream. Some preliminary work done here indicated that once a maximum allowable freezing point-acidity curve has been established for cream of a certain fat percentage, the addition of small amounts of neutralizer can be detected.

#### CONCLUSIONS

1. There is a direct relationship between developed acidity and freezing point which varies only slightly from one sample to another.

2. Neutralizers such as NaOH or  $MgCO_3$  do not lower the freezing point appreciably when used in necessary quantities.

3. Neutralizers such as  $Na_2CO_3$  or  $NaHCO_3$  lower the freezing point appreciably when used in necessary quantities.

4. Heating a neutralized sample tends to raise the freezing point slightly, but not enough to interfere with the accuracy of the test.

5. Neutralization of as little as .015 per cent can be detected depending upon the normal acidity of the neutralized sample and the kind of neutralizer employed.

6. Samples of milk with a low natural acidity (.10-.13 per cent) can be soured and neutralized as much as .075 per cent with NaOH without detection by this test.

7. After a sample has been neutralized, further souring will not affect the accuracy of the test.

8. The evidence available indicates that neutralization of milk can be detected by comparing the freezing point of the sample with the maximum allowable freezing point for a sample of that acidity.

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# AN IMPROVED AND MODIFIED EVENSON COLOR TEST FOR "REMADE MILK"

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Due to complaints by various health officials regarding mislabelling of remade milk an investigation was made of the methods for detection of remade milk in fluid milk.

Since the principle upon which Evenson based his studies of remade milk (1, 2, 3, 4) seemed the most promising of all the others listed in the literature (5, 6, 7, 8) it was studied first. Evenson's method had the added advantage of having been published and used in several similar instances. Several other methods based on different principles were tried but as the following modification of the Evenson method gave such promising results the others were discontinued.

Pipette 45 to 50 cc. of liquid milk into a centrifuge tube and precipitate the protein by the use of 5 per cent acetic acid. Centrifugalize and decant the supernatant liquid, wash the protein with distilled water, using a mechanical stirrer to break up the lumps. Again centrifugalize and decant. Extract fat from the protein by washing with 50 cc. portions each of acetone, ether, and acetone in the order named, using the mechanical stirrer to secure efficient disintegration of the particles of protein. Then continue the washing with distilled water, using the mechanical stirrer in every case until two successive washings give negative "Molisch Tests" for carbohydrates.

After two consecutive washings which show no presence of carbohydrates with the "Molisch Test" add approximately 15 cc. of a NaOH solution (225 gms. NaOH made up to 500 cc.) to the protein precipitate in the tube and mix with a stirring rod until all of the protein has been broken up and wetted with the NaOH. After two hours judge the tubes for their color against a known sample of liquid milk which has been carried through the above procedure. The samples containing the remade milk will show a distinctly yellow color while the other will not.

The judging of the colors of the protein should be made in daylight and not under electric lights.

## EXPERIMENTAL

Evenson in his article states that the method as he reported it would detect the addition of 10 per cent of remade milk in the liquid milk, but it

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was believed possible to make this test more sensitive if a more thorough washing of the precipitated protein could be perfected.

At the beginning of the experimental work the protein was precipitated out of 25 cc. of liquid milk in a 50 cc. centrifuge tube with a 5 per cent solution of acetic acid. The whey was then removed by centrifugalization and on each addition of wash water to the protein in the tube the protein was broken up by a stirring rod and centrifuged off. This method of washing was very effective and gave results more sensitive than Evenson's method of washing. However, it required some twenty to twenty-five washings in order to remove the last traces of lactose. The number of washings required so much time that some further modification was sought. This led to the use of a mechanical stirrer which could be used as a means of breaking up the curd. This materially assisted in washing the protein free of any uncombined lactose, for it breaks the particles up to a degree of fineness which assures complete removal. It was found that with the mechanical stirrer it was necessary to increase the size of sample used from 25 cc. to 50 cc., since more protein is lost during the washing.

TABLE 1

Liquid milk number	Per cent remade milk added	Color	Remarks
1	0	-	{ Did not show color with 5% but 10% showed color equal to 5% in other samples.
	5	+	
	10	++	
2	0	-	
	5	+	
	10	++	
3	0	-	
	5	+	
	10	++	
4	0	-	
	5	+	
	10	++	
5	0	-	
	5	-	
	10	+	
6	0	-	
	5	+	
	10	++	
7	0	-	
	5	+	
	10	++	
8	0	-	
	5	+	
	10	++	
9	0	-	
	5	+(?)	
	10	+	(Very slight yellow)
10	0	-	
	5	+	
	10	++	

- No yellow color.

+ Distinct yellow.

++ Very decided yellow color.

The mechanical mixer used in this laboratory was a Bodine laboratory motor with a rheostat. The motor was equipped with a stirrer made from a glass rod with a corkscrew twist on the end to give a mixing effect. This was so arranged on a ring stand that the 50 cc. conical centrifuge tube could be clamped in place during the mixing. If a 100 cc. tube with a flat bottom is used an ordinary malted milk mixer can be conveniently employed.

It was also found during the experimental work that by using a more concentrated NaOH solution (225 gms. NaOH made up to 500 cc.) instead of the 5 per cent solution, along with the mechanical mixing, that a 5 per cent addition of remade milk could be detected without any question. The depth of color, however, depends a great deal upon the degree of heat used in the processing of the powdered milk.

The samples of milk used in this work were pasteurized milk picked up in the Chicago area and were carried through the above procedure, using 5 per cent and 10 per cent additions of remade milk from various types of spray process dry milk. The results on a few of these samples are listed in Table 1.

It will be noted that in two of the samples listed above the five per cent addition did not show up as clearly as in the majority of cases. This undoubtedly is due to the degree of heat used in the processing of the powdered milk and the extent of reaction between the lactose and the protein. However, in all of the samples which were studied there was not a single sample of liquid milk which gave a positive test unless remade milk had been added.

A large number of raw milk samples picked up in another large city were run through the above test with equally good results.

#### DISCUSSION

Evenson stated that his method would detect the addition of 10 per cent of remade milk in the liquid milk. It was found, however, that by the previously outlined procedure of washing the protein and using the more concentrated NaOH solution, that a 5 per cent addition of remade milk could be detected without any question. The depth of color, however, depends a great deal upon the degree of heat used in the processing of the powdered milk.

The use of the mechanical mixer very materially assists in washing the protein free of any uncombined lactose. Its use is essential for breaking up the particles of protein to the degree of fineness which assures a complete removal of the lactose. Use of the "Molisch Test" for carbohydrates has been recommended to indicate when the precipitate is adequately washed. The Molisch Test may be eliminated after the operator has gained experience and knowledge enough to know the effectiveness of his washing. It was found in this laboratory following our system of washing that ten washings after the fat extraction were sufficient.

During the work on this method it was observed that old fluid milks showed a grayish brown coloration with the NaOH solution but this was distinctly different from the yellow coloration of the remade milk. Even though there is a distinct difference, the grayish brown color does interfere with distinguishing the presence of the lower percentages of remade milks. The color comparisons must also be made in daylight and not under electric lights.

The development of the yellow color usually begins to appear in a few minutes after the mixing with the NaOH and reaches a maximum in 1½ and 2 hours. It was found that the contrast is more striking at the two hour period than later when an off-color begins to develop in the curd. This off-color interferes with the readings. However, the contrast is still apparent at the end of 24 hours.

#### SUMMARY

Modifications have been made in Evenson's color test for "Remade Milk" which facilitates the detection of 5 per cent of remade milk in fluid milk.

The modifications made in the Evenson test are an improved method of washing the protein and the use of a stronger solution of sodium hydroxide which gives a more striking contrast between remade milks and natural milks.

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## RELATION BETWEEN RATE OF GROWTH AND MILK AND FAT PRODUCTION

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The efforts of research workers, breeders, and dairy farmers have been aimed for many years at ways and means of predicting production in dairy females before they were in milk. If some means could be devised to make such predictions at a period before the animals calved, a considerable saving would result and the breeding of dairy cattle would be facilitated. Prentice (1) has presented studies of the food consumption of calves which show correlation with their production at a later time. Turner (2) has intimated from his studies that the functioning of the pituitary might be closely related to milk production. It seemed, therefore, that a study of the rate of growth of young animals as correlated with the milk and fat production might yield results of interest. From the growth records of the University of Nebraska Holstein herd, 76 females were selected for this study. These animals were bred, reared, and tested for production in the herd under conditions that were as near comparable as possible. Furthermore, the animals were all closely related, since all were descended from families that have been closely related for about 30 years.

In making this study, three indices of growth were used, namely, gain in weight, increase in height at withers, and increase in chest girth. The birth measurements were compared with those at two years, and the percentage increase in the various measurements used as the rate of growth. Weights were determined by monthly weighings taken from three successive days and the average used. Measurements of height at withers were taken monthly with a calibrated measuring rod with a right-angle cross arm. The heights were measured in centimeters at the second dorsal vertebra when the animal was standing squarely on its four legs. The chest girth was taken in centimeters with a tape line around the chest, at the second dorsal vertebra at the top and just back of the elbows.

Table 1 presents the weights and measurements at birth and at two years, with the increase and percentage of increase for all Holsteins (3) compared with the average of the 76 females used in this study. It will be noted that there is a very close agreement between the standard as represented by all Holsteins in the table and the animals used in this study.

In order to study the possible relationship of gain in weight, increase in

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TABLE 1

*Measurements of Holstein females at birth and two years with percentage increases*

Type of measurement	Birth measurements	Two years measurements	Increase in measurements	Increase in per cent
Average of all Holsteins*				
Weight, lbs. . . . .	91	1108	1017	1117.6
Average 76 Females				
Weight, lbs. . . . .	87	1149	1022	1220.6
Average of all Holsteins*				
Chest girth, cm. . . .	76.2	188.0	111.8	146.6
Average 76 Females				
Chest girth, cm. . . .	77.9	190.2	112.1	144.1
Average of all Holsteins*				
Height at withers, cm.	73.7	133.4	59.7	81.0
Average 76 Females				
Height at withers, cm.	74.3	133.5	59.2	79.7

\* Reference 3.

height, and increase in chest girth, three factors which are used to measure growth, a study was made of each factor as it might be related to subsequent milk and butterfat production, both for the first lactation and for the lifetime average of lactations. Thus, in Table 2 the 76 Holstein females were arranged in classes in accordance with their percentage of gain in weight at two years over the birth weight. The class interval range was 50 per cent and the classes varied from 900-949.9 per cent to 1750-1799.9 per cent with a mean of 1220.6 per cent gain. When the various individual animals were assigned to the group into which each belonged because of percentage gain in weight, the average birth weight, the average weight at two years, the average milk and fat production for one year for the first lactation and for the lifetime average of lactations was determined for each class together with the range in production. To make all production records comparable they were corrected to maturity, 365 days, for three-time milking. Thus the range in milk production was from 11,996 to 26,670 pounds of milk, and in fat production from 476 to 878 pounds of fat for the first lactation, with a simple average of 18,283 pounds of milk and 666 pounds of fat. For the lifetime average of lactations the range was from 12,117 to 26,670 pounds of milk and from 476 to 873 pounds of fat with a simple average of 18,040 pounds of milk and 651 pounds of fat. No statistical computations were made, but an inspection of Table 2 will indicate quite definitely that there is no marked correlation between the percentage gain in weight during the first two years and the production of milk and fat.

Table 3 presents a like comparison based upon increase in height as measured in height at withers in centimeters. The average height at withers at birth was 74.3 cm. and at two years of age 133.5 cm., a percentage increase of 79.7. The individual females were grouped according to gain in height, with class intervals of two per cent. Thus, the lowest class interval was

TABLE 2

*Relationship of percentage of gain in weight two years over birth, with milk and butterfat production for first lactation and lifetime average*

Weight			Animals	Production							
Birth	Two years	Gain at two years over birth		First lactation				Lifetime average per lactation			
				Milk		Fat		Milk		Fat	
				Range	Average	Range	Average	Range	Average	Range	Average
<i>lbs.</i>	<i>lbs.</i>	<i>per cent</i>	<i>No.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
96	985	900-949.9	1	15,075	583	668-691	583	15,744-17,742	15,075	615-618	583
105.5	1121.5	950-999.9	2	18,018	679	590-680	679	15,124-17,315	16,743	569-624	616
95.0	1073.0	1000-1049.9	5	15,124-18,277	13,376	656-862	637	15,419-26,670	16,213	576-862	594
93.8	1100.6	1050-1099.9	9	16,602-26,670	19,782	476-806	707	13,088-22,080	19,153	494-775	680
90.5	1112.6	1100-1149.9	8	11,996-22,080	18,116	605-748	651	13,220-19,869	18,276	528-748	649
87.4	1108.6	1150-1199.9	5	14,903-19,869	18,125	501-816	664	12,117-21,123	17,732	488-752	658
88.4	1174.1	1200-1249.9	13	12,829-23,262	17,602	595-842	661	16,308-20,517	17,454	596-746	639
86.8	1197.3	1250-1299.9	8	16,655-21,135	18,901	476-878	680	13,995-20,468	18,265	476-762	661
81.9	1163.5	1300-1349.9	8	13,844-23,675	17,554	508-873	650	14,753-25,388	17,422	508-873	633
80.2	1167.6	1350-1399.9	8	14,686-25,388	18,131	562-739	659	15,310-23,276	17,658	535-741	643
80.0	1218.5	1400-1449.9	4	15,268-23,276	19,306		653		19,979		673
71.0	1123.0	1450-1499.9	1		15,019		512		15,596		528
85.0	1400.0	1500-1549.9	1		20,413		720		21,770		764
		1550-1599.9	0								
		1600-1649.9	0								
		1650-1699.9	0								
65.0	1193.5	1700-1749.9	2	16,630-21,277	18,953		713	19,013-23,222	21,142	702-803	752
60.0	1120.0	1750-1799.9	1		21,775		815		20,979		752
Averages, All Animals											
87	1149	1220.6		18,283	666			18,040			651

TABLE 3  
*Relationship of percentage gain in height at withers two years over birth, with milk and butterfat production for first lactation and lifetime average*

Height at withers		Production									
Birth	Two years	Gain at two years over birth	Animals	First lactation			Lifetime average per lactation				
				Milk		Fat	Milk		Fat		
				Range	Average		Range	Average		Range	Average
cm.	cm.	per cent	No.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
74.5	128.0	70-71.9	1	16,692-20,696	20,517	682	15,614-20,696	20,517			682
76.2	131.8	72-73.9	4	17,307-23,080	18,692	644	16,775-22,080	18,505		569-678	633
75.2	131.0	74-75.9	4	11,986-21,247	19,695	668-756	13,088-21,770	19,379		618-756	671
74.9	132.4	76-77.9	18	12,829-26,670	17,215	476-734	12,117-26,670	17,305		508-746	634
75.2	134.5	78-79.9	14	14,546-25,388	17,798	476-878	14,546-25,388	16,919		476-734	631
74.2	134.6	80-81.9	14	17,228-21,966	19,436	584-873	16,148-20,399	19,398		584-873	710
72.4	132.4	82-83.9	5	14,686-23,262	19,429	645-806	15,310-18,926	18,071		593-775	664
72.0	133.5	84-85.9	6	17,114-18,323	17,494	512-816	18,284-20,788	16,620		528-684	591
71.0	133.2	86-87.9	2	14,356-19,869	17,718	586-713	13,995-21,123	19,536		613-738	675
72.1	136.1	88-89.9	4		17,979	551-728		18,682		528-728	642
72.0	137.0	90-91.9	1		19,232			19,232			653
68.0	131.0	92-93.9	1		21,775			20,979			752
70.0	136.0	94-95.9	1		16,943			15,899			549
		96-97.9	0								
		98-99.9	0								
68.0	136.0	100-101.9	1		16,630			19,013			702
Averages, All Animals											
74.3	133.5	79.7		18,283		666		18,040			651

TABLE 4

*Relationship of percentage gain in chest girth two years over birth, with milk and butterfat production for first lactation and lifetime average*

Chest girth			Animals	Production					
Ave. at birth	Ave. at 2 yrs. of age	Gain at two years over birth		First lactation			Lifetime average per lactation		
				Milk		Fat	Milk		Fat
cm.	cm.	per cent	No.	Range	Average	Range	Average	Range	Average
				lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
84.5	187.3	120-123.9	3	17,315-21,247	19,477	591-748	17,315-21,247	19,477	591-748
80.0	181.5	124-127.9	2	18,236-20,517	19,76	680-682	16,783-20,517	18,650	624-682
82.0	188.6	128-131.9	9	13,944-26,670	19,475	554-873	15,124-26,670	19,595	575-873
82.3	192.6	132-135.9	3	18,277-23,276	20,749	656-739	15,614-20,696	19,862	569-739
81.4	194.0	136-139.9	10	12,829-22,080	17,686	501-756	12,117-22,080	17,466	488-756
77.6	188.0	140-143.9	6	16,602-18,567	17,381	637-693	16,148-21,123	18,615	593-746
77.8	191.8	144-147.9	15	11,996-21,675	18,117	476-878	13,088-21,770	17,823	494-764
75.9	189.0	148-151.9	11	13,844-21,837	17,612	476-806	13,844-23,222	17,428	476-803
76.0	192.6	152-155.9	5	14,903-23,262	18,687	605-816	13,220-20,788	18,071	528-738
72.8	187.6	156-159.9	5	13,663-19,726	16,852	512-684	14,640-19,766	16,359	559-709
75.0	198.0	160-163.9	1		17,410			17,410	
72.0	191.0	164-167.9	1		14,753			14,753	
69.5	186.5	168-171.9	2	16,630-21,966	19,298	684-718	19,013-19,073	19,043	615-702
69.0	189.0	172-175.9	3	14,356-21,775	17,989	551-815	13,995-20,979	17,194	526-752
Averages, All Animals									
77.9	190.2	144.1		18,283		666		18,040	651

70–71.9 per cent and the highest 100–101.9 per cent. The lowest average of any class interval group had a height at birth of 68.0 cm., the highest average of a class interval group a height of 76.2 cm. At two years of age, the lowest average of any group was 128.0 cm. and the highest was 137.0 cm. The milk and fat production ranges and averages were much the same as in Table 2. An inspection of this table reveals no apparent correlation between rate of increase or growth in height, as measured in height of withers from birth to two years, and the production of milk and fat.

In Table 4 the increase in girth between birth and two years as measured in chest girth is presented in relation to production. The average chest girth at birth was 77.9 cm. and at two years it was 190.2 cm., an increase of 144.1 per cent. The individual females were grouped according to percentage increase in chest girth in four per cent class intervals. The classes ranged from 120.0–123.9 per cent to 172.0–175.9 per cent. Chest girth at birth varied in class interval averages from 69.0 cm. to 84.5 cm., while at two years the lowest class interval average was 181.5 cm. and the highest 198.0 cm. Here, again, the production showed much the same range and average, and an inspection of Table 4 will very definitely indicate the lack of correlation between increase in chest girth and production.

#### SUMMARY AND CONCLUSIONS

An attempt was made to correlate rapidity of growth as indicated by gain in weight, by increase in height at withers, and by increase in chest girth from birth to two years, with milk and fat production for the first lactation and for the lifetime average of lactations. Seventy-six Holstein females in the University of Nebraska dairy herd were used. While the animals were apparently normal as compared with standards established in that herd, no apparent correlation was observable for any of the three measurements.

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# A MANGER FOR EXPERIMENTAL FEEDING

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Considerable difficulty has been experienced with losses of feed when conducting feeding experiments with dairy animals. When the amount of feed is restricted the head of the animal may be closely confined for a sufficiently long time to allow the animal to eat and by so doing insure against any considerable loss of feed; however, when the animal is fed to the limit of her capacity, eating requires considerable time and she must have enough freedom to insure comfort, therefore losses of feed are difficult to prevent.

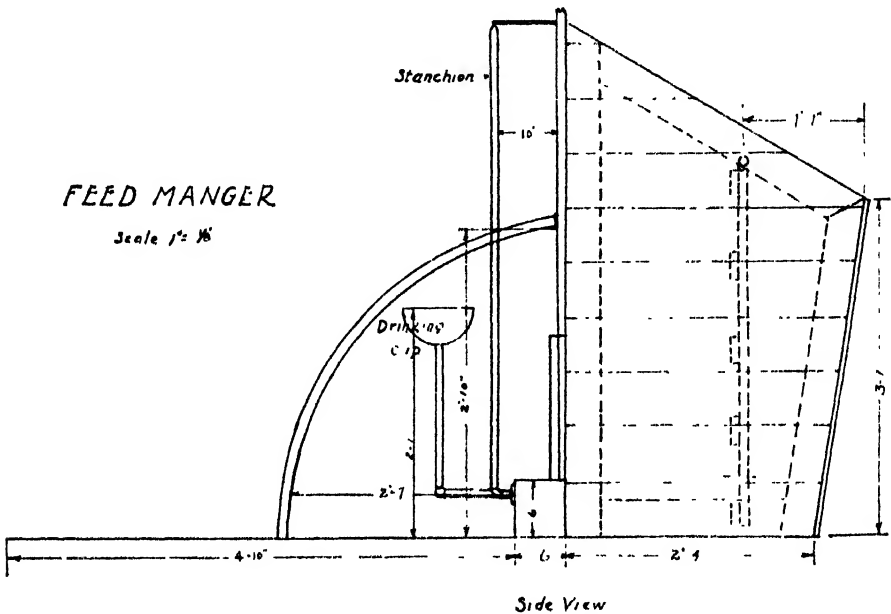


The accompanying diagram shows a manger that has proved satisfactory in use under the latter conditions. The mangers were built in sections long enough to provide individual mangers for three stalls. These were built and then set in place before the stalls. Two features distinguish these mangers from those that have been used commonly heretofore. First, a swinging rack or frame is suspended from a horizontal rod thirteen inches from the

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<sup>1</sup> Acknowledgment is gratefully made to Mr. A. G. Foster of the Agricultural Engineering Department for the accompanying sketch.

extreme front of the manger and high enough so that it will swing clear of the floor. The frame is made up of 1"×3" oak boards with the horizontal boards six inches apart. The hay is placed in the manger ahead of the frame and must be pulled through between the horizontals a mouthful at a time. The frame prevents the animals from throwing the hay over the front of the manger and is easily swung to the rear to clean the manger. Only one animal of twelve using the mangers draws enough hay through the frame to scatter appreciable quantities in the front part of the stall around her front feet, and even then the scattered material contains no leaves which drop in the manger. Two by six planks nailed to the manger divisions above the



concrete curbing are notched to form a V before the stanchion so that the cow may lie down comfortably with her head in the manger. The amount of hay strewn in the stall is reduced by having the rear of the manger built up higher. The higher rear manger partition also prevents the frame from swinging backward too far. The watering cups are located behind the manger and under the stall division, thus preventing spillage of water on refused feed which must be weighed back. The stanchions which are in use here may be adjusted according to the length of the animals to align the animals with the gutter. These must be adjusted to the extreme rear to allow the stanchion to swing freely behind the rear manger partition, if the cows are stabled for long periods of time.

These inexpensive mangers have largely eliminated experimental error due to losses of hay.

# FEED UNITS FOR LACTATION, WORKING MAINTENANCE, AND GAIN IN LIVE WEIGHT IN DANISH DAIRY COWS

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The Agricultural Experiment Station at Copenhagen, Denmark, has published (1) the results of an extensive series of feeding experiments, conducted on a number of privately owned farms, to ascertain the effect of light to heavy feeding on milk yield. The plane of feeding was varied with respect to total feed units with total protein constant, and with respect to total protein with total feed units constant. In general these experiments were planned, using the feeding standard,  $FU = .4FCM + (1.5 + .005W) + 4\Delta W$ , as a guide.<sup>1</sup>

The feed intake is reckoned in the Danish work in terms of feed units. One feed unit is the equivalent of one kilogram of barley. In American units we may say one feed unit = 1.72 pounds of digestible nutrients, and one feed unit per kilogram (FCM, etc.) = .78 pounds of digestible nutrients per pound (FCM, etc.).

The Danish feeding standard given above has three components:  $FU' = .4FCM$ ;  $FU'' = 1.5 + .005W$ ;  $FU''' = 4\Delta W$ . The  $FU''$  term is of special interest. It practically places the feed of working maintenance as proportional to the  $2/3$  power of live weight. The following numerical example will serve to show this:

	W = 400	450	500	550	600
$FU'' = 1.5 + .005W = 3.50$	3.50	3.75	4.00	4.25	4.50
$FU'' = .0635W^{2/3} = 3.45$	3.45	3.73	4.00	4.26	4.52

Thus, within the live weight range of its intended application the working maintenance standard is proportional to the  $2/3$  power of live weight.

## PURPOSE OF THIS PAPER AND RESULTS

It is proposed to apply the equation,

$$FU = aFCM + bW^c + d\Delta W \quad (2)$$

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<sup>1</sup> Symbols are used (except as noted) to apply to each experimental period for each cow, as follows:

- FU = nutrients intake, feed units per day.
- FU' = nutrients apportioned to lactation, feed units.
- FU'' = nutrients apportioned to maintenance, feed units per day.
- FU''' = nutrients apportioned to gain in weight, feed units.
- FCM = milk-energy yield, kilograms of 4 per cent milk per day.
- W = average live weight, kilograms.
- $\Delta W$  = gain in live weight, kilograms per day.
- n = number of cows or records.

to the data as given in Tables 15-40 of the Danish report. The published data include for each cow and each experimental period: the average feed intake in feed units per day; the average FCM yield in kilograms per day; the average live weight in kilograms (average of initial and final live weights); the average gain or loss in live weight in kilograms per day.

Equation (2) is fitted to these observations according to the method of

TABLE 1

*Feed units apportioned to lactation, working maintenance, and live-weight gain. Records of Red Danish cows from Royal Veterinary and Agricultural College, Copenhagen, in groups of 10 or 20. (See footnote, page 645, for explanation of symbols)*

Group No.	Sign of $\Delta W$	n	Live-weight limits, kg.	W	$FU' = aFCM_a$	$FU'' = bW_{1000} b$	$FU''' = d\Delta W_d$
1	-	10	381-418	407.1	.4993	5.94	1.536
2	+	20	396-423	413.4	.2850	10.56	.980
3	-	10	420-439	430.5	.6820	1.76	3.043
4	+	20	425-438	432.8	.3168	12.29	1.593
5	-	10	439-446	442.2	.5712	3.58	.053
6	+	20	439-447	443.3	.4590	6.70	4.060
7	-	10	446-450	448.2	.3480	10.68	2.158
8	+	20	447-460	453.4	.2671	12.64	.246
9	-	10	451-460	453.4	.3397	10.58	.186
10	-	10	460-465	462.8	.4556	7.35	.508
11	+	20	460-466	464.1	.5078	5.73	1.548
12	-	10	466-468	467.1	.3661	9.31	1.085
13	+	20	466-471	469.2	.4777	6.24	1.368
14	-	10	469-474	471.2	.6615	-.17	-1.866
15	+	20	472-476	473.4	.3839	9.08	1.241
16	-	10	475-481	477.5	.4332	7.06	-.556
17	+	20	476-481	478.2	.5656	5.15	-.910
18	-	10	482-486	483.6	.3030	10.09	.480
19	+	20	481-489	484.4	.5501	4.76	.003
20	-	10	487-493	489.7	.5419	7.21	1.104
21	+	20	489-495	492.3	.5346	4.93	.698
22	-	10	494-499	496.5	.5082	6.45	5.573
23	+	27	496-503	499.7	.4771	6.48	.480
24	-	10	500-505	501.6	.3904	7.81	-2.780
25	+	20	503-509	505.9	.4045	8.22	.653
26	-	10	505-513	509.8	.4271	8.40	2.757
27	+	20	509-513	511.1	.5119	5.84	-.030
28	-	10	513-518	515.2	.4411	7.26	-.439
29	+	20	513-519	515.8	.4127	8.36	-.192
30	+	20	519-526	522.5	.5527	4.32	2.577
31	-	10	518-527	523.7	.4112	7.04	-1.343
32	-	10	528-532	529.5	.5408	3.83	-.310
33	+	20	526-533	529.6	.4878	5.85	.101
34	+	20	533-540	536.6	.4635	6.98	-.706
35	-	10	533-541	537.4	.5151	5.62	1.805
36	+	20	540-551	545.6	.4638	5.55	3.185
37	-	10	542-550	546.0	.3492	9.07	-2.625
38	+	20	551-557	554.0	.4463	6.76	1.060
39	-	10	551-568	558.3	.4911	5.45	-.194
40	+	20	557-565	561.1	.3904	8.92	-1.159
41	+	20	566-590	575.6	.3545	9.72	.192
42	-	10	573-595	583.0	.4624	6.95	1.252
43	+	20	590-674	613.7	.5134	6.25	-.903
44	-	9	595-642	613.8	.2644	11.02	2.554

the preceding paper (2). The  $+\Delta W$  records (cows with final live weight equal to or greater than initial live weight,  $n = 447$ ) are arranged in order by  $W$  and divided into groups by successive 20's. The  $-\Delta W$  records (cows with final live weight less than initial live weight,  $n = 219$ ) are arranged in order by  $W$  and divided into groups by successive 10's. Each of these groups of records is fitted with the equation

$$FU = aFCM + K + d\Delta W \quad (1)$$

In equation (1)  $K$  represents  $bW^c$  of equation (2). Table 1 gives the constants of equation (1) for each group, but  $K$  is given, in the  $FU''$  column, as  $1000K/W$ . Figure 1 shows  $1000K/W$  plotted against  $W$ . The correlation between the two is  $r = -.00 \pm .10$ . There is no need to fit  $K = bW^c$ , for this zero correlation indicates that  $c = 1$  in equation (2). The  $a$ ,  $b$ , and  $d$  constants of equation (2) are taken to be, respectively, the average of the  $a$ ,<sup>2</sup>  $b$ , and  $d$  values of Table 1. This gives

$$FU = .451FCM + .00713W + .68\Delta W \quad (3)$$

#### DISCUSSION

It is of interest to compare equation (3), representing Red Danish cows in farm herds in Denmark, with the corresponding equation representing Guernsey, Holstein, and Jersey cows in Experiment Station herds in the United States (2). Equation (3) may be converted to terms of digestible nutrients and pounds by substituting  $DN$  for  $FU$  and multiplying through on the right by .78.

Comparison of the feeding standards, indicated by the Danish and American data, follows:

$$\text{(Danish)} \quad DN = .35FCM + .006W \quad (4)$$

$$\text{(American)} \quad DN = .28FCM + .009W \quad (5)$$

in which all terms are expressed in pounds (instead of kilograms). According to these results the Danish cow, as compared with the American, requires  $\frac{1}{4}$  more digestible feed energy for lactation to produce a unit of milk energy, but  $\frac{1}{4}$  less feed for maintenance per unit live weight. A difference of this magnitude, if real, commands attention.

Can it be that the Red Danish cow is more sluggish and expends less energy in muscular and general body activity, thereby reducing the energy cost of maintenance? (The Danish data include ages from 2.56 years to 14.32 years, with one cow 24 years old, while in the American data cows under 5 years of age are excluded.) If we could substitute  $.006W$  for  $.009W$  in equation (5) it would mean a saving of 19 per cent in the overall feed cost of producing milk in the case of a 1000-pound cow giving 25 pounds of 4 per cent milk per day.

<sup>2</sup> The correlation between  $a$  and  $W$  of Table 1 is  $r = -.06 \pm .10$ , and the coefficient of regression of  $a$  on  $W$  is  $-.00011 \pm .00019$ .



use of inadequate data or methods? Some of the limitations in this regard have been previously mentioned (2, 3). As the case stands it seems that feeding standards for cows in milk need to be adapted to the particular breed or kind of cow with which we are dealing. In practice the feeding standard affords the basis of formulating a trial ration. Adjustment may have to be made for the individual cow, according to her response in milk yield and gain in live weight. It is evident that while  $\Delta W$  and  $d\Delta W$ , as used in the equations, are small in magnitude they may be large in physiological significance. Note the prevalence of minus  $d$ 's in Table 1 and their drift to the larger  $W$ 's. Undoubtedly more trustworthy results could be obtained from the present method of analysis if the experiments were designed for the purpose. Nevertheless, the data and analysis, as they stand, warrant tentative conclusions.

#### SUMMARY AND CONCLUSIONS

Six hundred sixty-six records of Red Danish cows on farms in Denmark are analyzed to apportion the nutrients consumed between lactation, working maintenance, and gain in live weight.

A feeding standard derived from the records disagrees in some respects with the standard which guided the feeding of the cows. Notably, the standard used assigns nutrients for working maintenance proportional to the  $\frac{2}{3}$  power of live weight, while the present analysis of the records shows the nutrients for working maintenance are proportional to live weight.

The present standard from records of Red Danish cows, compared with the standard similarly derived from records of Guernsey, Holstein, and Jersey cows in Experiment Station herds in the United States, shows a great difference, as follows:

$$\begin{array}{ll} \text{(Danish)} & \dots \dots \dots DN = .35FCM + .006W \\ \text{(American)} & \dots \dots \dots DN = .28FCM + .009W \end{array}$$

where DN is digestible nutrients consumed, pounds per day; FCM is milk energy yield, pounds of 4 per cent milk per day;  $W$  is live weight of cow, pounds. It is tentatively concluded that the Red Danish cow is not so well developed as a milking cow, requiring more feed energy for lactation per unit of milk energy produced, but, on the other hand, is a more sluggish cow requiring less feed energy for working maintenance per unit live weight. A feeding standard for cows in milk needs to be adapted to the particular breed or kind of cows for which it is used.

The Danish and American data agree in indicating that working maintenance is proportional to live weight, and not to a fractional power of live weight.

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# THE USE OF RECORDS IN EVALUATING THE INHERITANCE OF COWS AND IN THE PROVING OF BULLS

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With the Herd Improvement Registry test of all the dairy breeds continually increasing and with the Dairy Herd Improvement Association program maintaining its popularity, there has been increasing attention devoted in recent years to lifetime production records. In some instances, national Breed Associations have granted special honor to cows that during their entire lifetimes, produced certain quantities of milk and butterfat. Cows that have produced one-hundred thousand pounds of milk in their lifetimes, often have been given special awards and in local associations and shows, liberal recognition has been bestowed upon lifetime champions. Frequently, special classes have been offered for "Ton" cows and for "Two Ton" cows, that is, cows which have produced a ton or two tons of butterfat during their lifetimes. With this increasing attention being given to lifetime production, the tendency has been in some instances to discount and discredit individual records.

There is no question but that any cow which has produced one or two tons of butterfat during her lifetime is deserving of recognition as she has undoubtedly been a profitable dairy animal. It is true that cumulative production indicates that a cow has been a financial asset to the herd. In view of this emphasis on lifetime yields, it seems that a study of the contribution such cows have actually made toward herd and breed improvement is needed. Is it possible that as a basis for genetic study, the use of lifetime averages tends to reduce variation and to distort to some extent the inferences that would be drawn from such data? With the high probability that over a period of years some uncontrollable factor will interfere with the producing ability of a cow, is there a tendency to introduce this environmental influence if average records are used? During the past decade, the results of much research have been published indicating that the production record of a cow considered alone is a poor measure of the cow's possible transmitting ability. Most of the publications on this subject have considered only one record.

The question now arises, is the average of two, three, four or more records any better index as to a cow's transmitting ability than is one record? There are also other related questions that are pertinent at this time. If dam and daughter comparisons are used in attempting to evaluate the transmitting ability of a bull, should the highest records of each dam and daughter be compared; or should the two year old records of the dam and daughter be

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compared; or should a comparison be made between the average of all the records completed by each dam and each daughter? The latter method is the one most frequently used in Dairy Herd Improvement Association work. Then, what is the correlation that exists between one record and another record made by the same cow? Does the first record completed by a cow give much indication as to what she will produce in subsequent lactations barring illness or accident? If a cow has completed several records, does the first lactation record or does the highest record give a better indication as to the lifetime production?

In an attempt to throw further light on these questions, the Register of Merit and Herd Improvement Registry records of Jersey cows have been studied. In analyzing the records, it was first attempted to determine the repeatability of records made by Jersey cows. There were 197 cows which had completed five or more 305 or 365 day Register of Merit records. First, each record completed by each cow was converted to a mature 365 day equivalent basis using the age conversion factors previously tabulated and published by the American Jersey Cattle Club. Then the correlation between the first and second records completed by each cow was determined. Similarly, the correlation coefficients were determined between the second and third, the third and fourth, and the fourth and fifth records for each cow. Next, a comparison was made between the first record completed by each cow and the average of the next four records. Likewise, correlations were tabulated between the first record completed and the average of all five records and lastly, the highest record of each cow was compared with the average of all

TABLE 1  
*Correlation coefficients between records made by the same cow*  
(197 cows with 5 or more R. of M. records used)

Comparisons and correlations made	Coefficient of correlation	No. of + variations	No. of - variations	Av. + and - variation
Comparison of 1st R. of M. record with 2d record	$+ 0.71 \pm .024$	65	132	+ 57 - 103
Comparison of 2d R. of M. record with 3d record	$+ 0.77 \pm .020$	109	88	+ 71 - 80
Comparison of 3d R. of M. record with 4th record	$+ 0.69 \pm .025$	99	98	+ 85 - 86
Comparison of 4th R. of M. record with 5th record	$+ 0.59 \pm .031$	98	99	+ 102 - 94
Comparison of 1st R. of M. record with average of next 4 records	$+ 0.62 \pm .030$	72	125	+ 57 - 103
Comparison of 1st R. of M. record with average of all 5 records	$+ 0.75 \pm .021$	74	123	+ 46 - 86
Comparison of highest R. of M. record with average of all 5 records	$+ 0.92 \pm .007$	197	0	+ 99 - 0

five records. Also, the average variations were determined for each comparison. The results are shown in Table 1.

The results of the first four comparisons show that in comparing individual records, the greatest correlation exists between the second and third records completed. Also, the average variations are less between the second and third records. All of the coefficients of correlation are very good showing a relatively high degree of repeatability between normal records made by the same cow. The last three comparisons are significant. Less correlation existed between the first record completed by a cow and the average of the next four records, than between the first and second records. When the first record was correlated with the average of all five records, the correlation was still higher for, in this instance, the first record was included in making the average for the five records. The correlation between the highest record completed and the average of all five records is extremely high and indicates that the highest record completed by any cow gives a very good estimate as to what the lifetime average will be provided the cow is kept on test continuously and encounters no disease or misfortune. It should be mentioned that in 147 instances of the 197 cows used for analysis, the first Register of Merit records were begun at under three years of age, indicating first lactation records. In nearly all cases, the subsequent records were made in consecutive lactations.

Next, exactly the same comparisons were made using 166 cows that had finished five or more consecutive, complete Herd Test lactations. These results are shown in Table 2.

TABLE 2

*Correlation coefficients between consecutive Herd Test records made by the same cow  
(166 cows with 5 or more Herd Test records used)*

Comparisons and correlations made	Coefficient of correlation	No. of + variations	No. of - variations	Av. + and - variation
Comparison of 1st Herd Test record with 2d record	+ 0.78 ± .021	70	96	+ 111 - 60
Comparison of 2d Herd Test record with 3d record	+ 0.80 ± .019	89	77	+ 75 - 87
Comparison of 3rd Herd Test record with 4th record	+ 0.75 ± .023	102	64	+ 98 - 54
Comparison of 4th Herd Test record with 5th record	+ 0.83 ± .016	121	45	+ 78 - 76
Comparison of 1st Herd Test record with av. of next 4 records	+ 0.80 ± .019	105	61	+ 93 - 49
Comparison of 1st Herd Test record with av. of all 5 records	+ 0.88 ± .012	104	62	+ 74 - 39
Comparison of highest Herd Test record with av. of all 5 records	+ 0.92 ± .008	166	0	+ 98 - 0

The correlation coefficients in this table differ just slightly from the similar comparisons made on the cows with Register of Merit records. In most instances, the correlation coefficients are a little higher than shown in Table 1. This result was not unexpected for most Herd Test records are made on a lower level of production than Register of Merit records, which naturally tends to reduce the variation from the individual records and this reduction in variation may tend to increase the correlations. It will be observed that all of the correlations are exceptionally good and that the highest record completed shows a remarkable correlation with the average of all five lactations.

The results thus far have shown that the highest record completed by a cow is a good indication of her potential lifetime production, barring accident, disease, or other misfortune. However, in addition to indicating the economic worth of a cow as a producer in the herd, production records are also used in the proving of sires and in estimating a cow's transmitting ability. The great lifetime producing cows with a number of records to their credit and high lifetime production totals, have been profitable dairy animals but it is important to learn their contribution to their progeny and descendants. Have they made any more contribution than cows which have completed only one or two high records? It is undoubtedly true that there are instances of cows having been injured physically, due to over feeding while making a record, and it is also true that some of the cows which have completed exceptionally high records have not been tested again. Consequently, in such instances, these cows are credited with only one record. In most cases, nothing is known as to why these cows were not tested again and not given the opportunity to see what they might have done during an entire lifetime. Sometimes, such cows have been held up to ridicule as having made no contribution at all to the breed, while other cows that have not been able to produce nearly as much in a single lactation, but which having been tested year after year and amassing fair lifetime yields, have been pointed to with pride.

It seemed worth while to study the transmitting ability of the lifetime production champions and also the transmitting ability of cows which for some reason had only been tested once but which had completed an exceptionally high yield. In the Jersey breed, there are 176 cows that have completed five or more 305 or 365 day Register of Merit records and which also have at least one officially tested daughter. This group was divided into two divisions. The first division of 87 cows with five or more records had lifetime productions of less than three-thousand pounds of butterfat. Two comparisons were made on this group. First, the lifetime production was divided by the number of lactations giving the average yield for each record. This average yield for the records completed by each cow was then compared with the average yields of the daughters' records. The average of all the records

completed by each dam was 485.51 pounds of fat and the average of all the daughters' records was found to be 558.26 pounds of fat. In the second comparison, the highest record completed by each of the dams was compared with the highest record completed by the daughters. When the highest record of each dam was selected and the average obtained for the 87 cows, it was found to be 590.52 pounds of fat. The highest records of the daughters when averaged was found to be 583.81 pounds of fat. In this analysis, it should be mentioned that the average production of all the mature (6 to 10 years) 365 day Register of Merit records that have ever been completed is 556.50 pounds of fat.

The second group consisted of 89 cows with five or more Register of Merit records but with lifetime totals exceeding 3,000 pounds of butterfat. All 89 cows had officially tested daughters. The same two comparisons were made on this group as with the first group, namely, the average of the dams' records was compared with the average of the daughters' records and then the highest record of each dam was compared with the highest records completed by the daughters. The average of all the dams' records was 622.27 pounds of fat and the average of all the records completed by the daughters was 612.07 pounds. When the highest record completed by each dam was selected and these high records averaged for the 89 dams, the result was 777.87 pounds of butterfat. This figure was then compared with the average of the highest records completed by all the tested daughters which was found to be 647.13 pounds of butterfat. It is obvious that the daughters of the cows with lifetime records of over three-thousand pounds of butterfat have exceeded the breed average considerably in production, while the daughters of the cows with lifetime records totalling less than three-thousand pounds of butterfat have just equalled the average for the breed.

It was then ascertained that to October 1st, 1932, a total of 219 cows had been tested just once for the Register of Merit and had completed a single record exceeding 740 pounds of butterfat. No information is available as to why these cows were never entered on test again. It may be that in some instances, the cows did not calve normally again, although it was ascertained that 115 or fifty-three per cent of these cows did calve immediately after the completion of their records and qualified for Class AA or Class AAA and in 172 instances or seventy-nine per cent, calves born after the completion of the record were registered from these cows. There were 118 or fifty-four per cent of the 219 cows with two or more registered progeny born after the dam completed her high record. It seems obvious that in most instances these cows were owned by breeders who did not follow a continuous year after year testing program as was the case with the owners of the preceding group of lifetime champions. This is indicated by the fact that only 88 of these cows with one record exceeding 740 pounds of butterfat have officially tested daughters. The records of these 88 cows were averaged and the result was

790.72 pounds of fat. It will be noted that this average is just slightly higher than the average of the highest records completed by each of the second group of great lifetime producers. Next, the records of the daughters of these 88 cows were studied and two tabulations made. First, all of the records completed by each daughter were averaged and the average was found to be 639.53 pounds of fat. In the second tabulation, the highest records of all the daughters were averaged and the result was 651.43 pounds of butterfat. These tabulations are all given in Table 3.

TABLE 3

*High lifetime record cows and high individual record cows and the records of their progeny*

	Average of all records completed by each cow	Average of highest rec- ords com- pleted by each cow	Average of all records completed by all daughters	Average of highest rec- ords com- pleted by all daughters
87 cows with 5 or more R. of M. records totalling less than 3000 lbs. of fat and having tested daughters	485.51	590.52	553.26	583.81
89 cows with 5 or more R. of M. records totalling more than 3000 lbs. of fat and having tested daughters	622.27	777.87	612.07	647.13
88 cows with only one record and that record above 740 lbs. fat, and having tested daughters		790.72	639.53	651.43

Of principal interest is the fact that the daughters of these cows with only one high record show a slightly higher yield than do the daughters of the cows with lifetime records exceeding three-thousand pounds of butterfat. Apparently the daughters of the cows that have completed only one extraordinarily high record have themselves produced as well or better than have the daughters of the cows which during their lifetimes have produced more than three-thousand pounds of butterfat.

Previous work by numerous investigators has shown that the correlation existing between a dam's record and the daughter's record is relatively low. Correlation coefficients between dam and daughter records as published by Gowen (1), Turner (2), Gifford and Turner (3), and Smith, Scott and Fowler (4), range between +0.259 and +0.42. The following two tables illustrate the relationship existing first, between the average records of the dams with the average of their daughters' records and second, between the highest record of each dam and the highest records of her daughters.

In both of the preceding tables the relationship seems to be about the same and while there is some correlation existing between the records of the dams and the records of the daughters, the variation in the yield of each group of

TABLE 4

*Comparison of average records of cows having 5 or more records with the average records of their tested daughters*

Production divisions of dams	No. of comparisons	Av. yield of dams	Av. yield of daughters
700 lbs. and over	16	761	640
650 to 699 lbs.	14	675	617
600 to 649 lbs.	24	622	629
550 to 599 lbs.	27	573	607
500 to 549 lbs.	41	523	560
450 to 499 lbs.	30	476	548
400 to 449 lbs.	17	432	551
Under 400 lbs.	7	359	550
Total and averages	176	554	585

daughters was quite pronounced. This is best indicated by the fact that the coefficient of correlation between the highest records of the dams and the highest records of the daughters was found to be  $+0.29 \pm .047$ , while the correlation between the average records of the 176 dams and the average yield of their tested daughters was found to be  $+0.30 \pm .046$ . These correlations are most significant. While they are both quite low, they are practically the same and indicate that the highest record of a cow gives about as much information concerning a cow's possible transmitting ability for production to her

TABLE 5

*Comparison of highest records of cows having 5 or more records with the highest records of their tested daughters*

Production divisions of dams	No. of comparisons	Av. yield of dams	Av. yield of daughters
900 lbs. and over	12	979	688
850 to 899 lbs.	11	870	665
800 to 849 lbs.	14	823	663
750 to 799 lbs.	14	767	601
700 to 749 lbs.	24	725	672
650 to 699 lbs.	23	670	581
600 to 649 lbs.	31	626	591
550 to 599 lbs.	19	577	588
500 to 549 lbs.	15	530	544
Under 500 lbs.	13	457	579
Total and averages	176	685	614

daughters, as does the average of a series of records completed during an entire lifetime.

An item of interest in this connection is that the 197 cows with five or more Register of Merit records have an average of 6.05 registered progeny per cow, to date. The 219 cows with only one high Register of Merit record have an average of 4.70 registered progeny each, to date. It is realized that in both cases, these averages of progeny are not complete for in many instances

calves are still being registered from these cows and also very often male calves are not registered. In addition, in a number of cases, calves may have died before being recorded.

As a supplement to the data already presented, a study was made of the first calf heifers of the breed which had completed exceptionally high records. Prior to 1933, a total of 318 heifers starting test at two years nine months or under, have completed a Register of Merit record in excess of 600 pounds of butterfat. Of these 318 high record first calf heifers, 172 completed second records and 70 have completed three or more records. The first and second records were compared and the correlation coefficient was found to be  $+ .55 \pm .036$ . This correlation while good is somewhat lower than the correlations between the first and second records shown in Table 1 and Table 2. When the records were computed to maturity, the first records of the 172 cows averaged 890 pounds of fat and the second records averaged 833 pounds of fat. It was then determined that 127 of these high record first calf heifers have tested progeny. The records of the cows and the records of their progeny were converted to a mature yearly basis. When the highest record of each cow was compared with the highest record of the daughters for the entire group, the correlation coefficient was found to be  $+ 0.30 \pm .055$ . This correlation is in agreement with the previous correlations between the highest record of the lifetime dams and daughters and the average records of the lifetime dams and daughters. The following table also helps illustrate the relationship existing between the highest records of these cows compared with the highest records of their tested daughters.

TABLE 6

*Comparison of highest records of (high yielding) heifers with highest records of their tested daughters*

Production divisions of dams	No. of comparisons	Av. yield of dams	Av. yield of daughters
1000 lbs. and over	22	1071	777
950 to 999 lbs.	15	970	770
900 to 949 lbs.	19	918	708
850 to 899 lbs.	29	870	704
800 to 849 lbs.	34	831	688
Under 800 lbs.	8	782	606
Totals	127	890	715

Twenty-eight of these high record heifers were the dams of "tested" bulls. The records of the twenty-eight dams averaged 957 pounds of fat and the records of the daughters of the twenty-eight sons averaged 636 pounds of fat. In the preceding group of cows with five or more records, nineteen were the dams of "tested" sons. The highest records of the dams averaged 864 pounds of fat and the sons' daughters' records averaged 627 pounds of

fat. The 317 high record first calf heifers have an average of 4.37 registered progeny each, to date, which is slightly less than the two preceding groups studied.

#### SUMMARY

In summarizing the results of both phases of the investigation, it seems apparent that given the opportunity and barring illness or injury, a cow capable of completing one good record should be able to complete a number of lactations with good yields and thus finish a lifetime with a creditable total production. This is not in any sense an argument against continuous year after year testing. Continuous testing is needed to prove bulls at the earliest possible age. Several records are often needed on many cows to secure one complete lactation in which everything including health, feeding and management, and climatic conditions are fairly normal. Records made following abortions, attacks of bloat, milk fever and mastitis infection, do not give a true picture of a cow's ability at all. Neither are the records made in some sections of the drouth area during recent years, a fair measure of a cow's actual ability. Furthermore, only by continuous testing can a breeder tell definitely when a cow has ceased to be a profitable member of the herd.

Because of the necessity to assay the transmitting ability of bulls at the youngest possible age, it is impractical to compare lifetime records of daughters with the lifetime records of their dams. If we compare the average of several records on the dams with the first lactation records completed by the daughters, the results may be misleading. In selecting bulls, if the practice of comparing the highest record of the daughter with the highest record of the dam is used, no allowance is then made for the probability that the daughters will better their early records in later life and this method should improve the chance of a breeder being correct in selecting proved sires. In other words, an additional safeguard is furnished by insisting that the daughters of a bull in one or two trials produce as well or better than their dams were able to do in perhaps five or six trials.

If a cow has completed one record of 600 or 700 pounds of butterfat, she obviously possessed the inheritance to produce that amount in a lactation, unhampered by illness, poor management, or drouth conditions and consequently it seems that one record, and preferably the highest record, is suitable for evaluating the sire's ability and in trying to measure the cow's transmitting ability. Breeding operations will be seriously handicapped if breeders are forced to wait until cows have finished a series of four or five or more records, in order to obtain worth while information as to the sire's ability or the cow's own possible transmitting ability.

It also seems apparent from the data presented, that neither the highest record nor the average of several records gives a great deal of information

concerning the cow's transmitting ability as measured by the correlation with the daughter's production. To attempt to measure this, additional information is essential, such as the records of a cow's sisters and the records of her daughters. In a previous paper by the author (5), it was concluded that the record of a cow together with the records of her daughters and the records of her sisters did give a fair estimate concerning her transmitting ability.

Lifetime productions are largely influenced by longevity, opportunity, and perhaps to a certain extent, good fortune. Little if any data have been published concerning the inheritance of longevity. Unfortunately this is often not determined by nature. A high percentage of cows culled from the herds are removed due to disease infection. Is there any reason to believe that a cow, descending from a line of long-lived ancestors, is more immune to mastitis, Bangs' infection, etc., than is the daughter of a cow living only long enough to complete one or two lactations? Udder attachment is also another factor affecting the length of time cows remain in herds and it may be that some cows with a high inherited producing ability have not inherited a sufficiently strong udder attachment to keep the udder from breaking away and becoming pendulous. However, feeding and management may also be partly responsible for udder troubles. The inheritance of high milk producing ability, longevity, breeding efficiency, disease resistance and strength of udder attachments, all contribute to the end product of profitable lifetime production. Yet, it seems that these are separate problems in themselves and the selection of the highest record of an animal is the best index as to that animal's actual inherited production capacity under normal conditions.

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## FEED FLAVORS IN MILK AND MILK PRODUCTS

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The producer of milk is always confronted with the problem of preventing sour milk. With the increased consumption of milk and milk products has come the demand that milk not only be sweet but that it have a pleasing flavor. Milk containing an abnormal flavor is rejected by dealers and consumers. Dairymen are giving considerable attention to the prevention of losses due to sour milk. They too rarely recognize, however, that the production of milk containing objectionable flavors not due to souring is causing an annual loss probably greater than that from sour milk.

The fact that the feed consumed by the cow may be a contributing cause of abnormal flavors in milk has long been recognized. As early as 1829 William Harley (1) described a method for "preventing milk from tasting of turnips." He also observed, "It is chiefly common turnips and cabbages that give the strong taste to milk and butter." Many other early references are available dealing with the effect of feeds on the quality of milk. Almost without exception, in these early studies, quality was based on the chemical constituents of the milk. The effect of feeds on the flavor of milk was overlooked, or, if noted, dismissed with a sentence or two.

Although some work was reported prior to that reported by Gamble and Kelly (2), apparently it was the systematic study by the latter investigators on the effect of silage on the flavor and odor of milk that initiated recent interest in the subject. They reported a wide variation among individual normal cows in the flavor and odor of the milk produced. Cows receiving the same feed and care produced milk that ranged in flavor from pleasing to objectionable. Roadhouse, Regan, and Mead (3) confirmed the fact that there is a marked difference in the flavor of milk of individual animals and later Roadhouse and Koestler (4) reported on the causes of these variations in the flavor of milk from individual cows.

Gamble and Kelly (2) showed that in feeding corn silage before milking, when as little as 10 pounds was given at a feeding, the milk took on, through the body of the cow, a faint feed flavor and odor. As the quantity was increased to 30 pounds at a feeding, the degree of silage flavor and odor was likewise increased. This confirmed the work of Knisely (5), who reports that milk from cows fed corn silage has a more pronounced odor than milk from cows fed hay. King (6) also stated "It was demonstrated beyond

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question that when silage is fed a short time before milking, a sweetish odor is imparted to milk."

Gamble and Kelly (2) showed further that when as little as 30 pounds of corn silage was fed daily, in two feedings immediately after milking, the milk showed a slight feed flavor and odor; and that when more than 40 pounds was fed, the milk carried a slight silage flavor and odor continuously. Henry and Morrison (7) report that as the silage-feeding period progressed the effect of the silage became less and less apparent in the milk. Gamble and Kelly (2) showed that this applied, however, only when less than 35 pounds was fed per cow per day; it was shown that when over 40 pounds was consumed, the sweetish feed flavor could always be detected. From their work with corn silage they concluded that: (1) When silage is fed 1 hour before milking its taint is discernible in the milk. (2) Not over 15 to 25 pounds of corn silage can be fed twice daily after milking without imparting a discernible flavor and odor to the milk. (3) Silage should be fed immediately after milking.

In experiments with alfalfa, sweetclover, and soybean silages Gamble and Kelly (2) showed that legume silages should also be fed only after milking and then in quantities of not more than 15 pounds to a feed twice daily if milk reasonably free from feed taints is to be obtained. In regard to soybean silage, Woll and Humphrey (8) stated that satisfactory dairy products could not be made when cows were fed this silage, and Woodward and McNulty (9) reported that silage made from clover, while palatable, has an objectionable odor necessitating care in feeding to avoid tainting the milk.

Russell (10) states: "As milk is exposed during the milking process, and very often after its withdrawal to an atmosphere that is liable to contain odors of an undesirable character, it is not surprising to note that it may thus contract flavors by direct absorption." Ritland (11) expressed the opinion that the flavor noted in milk of cows fed turnips is due entirely to the absorption by the milk of volatile ingredients of the turnips. Farrington (12) says, "It has repeatedly been proved that silage can be fed to dairy cows without tainting the milk, butter or cream, in the slightest, but unless certain precautions are taken to prevent this, the cream or butter may be so tainted with silage smell that many customers will refuse to use it. The success of feeding silage depends almost entirely upon the disposition of the man feeding it, to constantly keep the air in the stable free from silage smell."

As a result of trials in which silage was spread on the floor underneath two cows in a stable with the doors and windows tightly closed, thereby exaggerating barn-air saturation, Gamble and Kelly (2) showed that silage-intainted barn air may have some effect on the flavor and odor of milk under extreme conditions, but concluded that the effect would be relatively under average conditions.

They further showed that careful and prompt aeration of the warm milk will remove silage flavors and odors permanently, if the milk was only slightly tainted, and will reduce the degree of the silage flavors and odors if the taint was more pronounced. This is in agreement with Marshall (13), who stated, "Odors and taints resulting from aromatic foods, physiological processes, and disease processes may be greatly reduced permanently," by aeration.

Other facts brought out by Gamble and Kelly (2) were: (1) Feeding moderate quantities of corn silage after milking and prompt aeration of the milk may in some cases actually improve the flavor of milk that would otherwise have a flat or insipid taste. (2) While silage odors in the barn air have only a slight effect on the flavor and odor of milk, it is best to provide adequate ventilation and to practice other sanitary measures to insure the finest possible flavors. (3) The feeding of badly decomposed or moldy silage imparts undesirable flavors to milk. (4) Cream from silage-tainted milk possesses and retains silage flavors and odors to a greater extent than the milk from which it is taken. (5) Condensed milk made from silage-tainted milk has a less perceptible silage flavor and odor than the milk from which it is made.

Babcock (14-18) confirmed the work of Gamble and Kelly (2) in that he found: Feed flavors are more pronounced in the cream than in the milk from which the cream is taken. Proper aeration reduces strong off flavors and odors in milk caused by feeding highly flavored feeds, and some of the slight off flavors and odors may be eliminated. Even highly flavored feeds may be fed immediately after milking without seriously affecting the flavor of the milk produced at the next milking.

He showed that when fed to dairy cows 1 hour before milking, green alfalfa, cabbage, turnips, rape, and kale seriously affect the flavor and odor of milk. Green rye, green cowpeas, potatoes, dried beet pulp, and carrots affect milk only to a slight degree. Green corn, green oats and peas, green soybeans, pumpkins, and sugar beets have practically no effect on the flavor and odor of milk. When dairy cows were fed 1 hour before milking time and consumed 15 pounds twice daily of those feeds that were found to affect seriously the flavor of milk, objectionable flavors and odors were produced in the milk. Increasing the consumption of these feeds to 30 pounds twice daily greatly increased the intensity of the abnormal flavors and odors. When these feeds were fed in quantities up to 30 pounds twice daily immediately after milking, they had practically no effect on the flavor and odor of the milk produced at the next milking. In fact, in the case of green alfalfa, it was shown that changing the time of feeding from 1 hour before milking to 3 hours before milking, decreased the intensity of the abnormal flavor, and feeding 5 hours before milking practically eliminated it. On the other hand, large quantities of feeds like cabbage and turnips, even though fed 12 hours before milking, still produced a strong off flavor and odor in the milk.

ately after milking, may at times slightly taint the flavor of the milk produced at the next milking. These taints, however, are slight and would seldom be noticed by the average consumer. Feeds that had only a slight effect when fed before milking had no detrimental effect when fed after milking.

In order to show more conclusively that feed flavors enter milk mainly through the body of the cow and to determine the time required for flavors to enter the milk, Babcock (19) conducted feeding experiments with garlic. This work showed that garlic flavor and odor can be detected in the milk when the milk samples are taken 1 minute after garlic is fed. The intensity of the garlic flavor increases as the time interval between feeding the garlic and taking the milk samples increases, until at 10 minutes a high degree of intensity is reached. Garlic flavor is present to a very objectionable degree in milk from cows that have consumed one-half pound of garlic 4 hours before milking. Milk drawn 7 hours after the cows consume one-half pound of garlic is practically free from garlic flavor. Strong garlic flavor is found in milk drawn 2 minutes after the cows inhale garlic odor for 10 minutes and practically disappears in 90 minutes after such inhalation. Garlic odor is readily perceived in samples of blood drawn 30 minutes after the cows are fed 2 pounds of garlic tops and strong garlic odor is present in the blood drawn 45 minutes after such feeding, indicating that the flavor is transmitted by the blood to the udder.

His work with bitterweed (20) further confirmed the fact that flavors enter milk mainly through the body of the cow. This weed is frequently found in southern pastures and, although it is practically odorless, it imparts its flavor to the milk when the cows eat it. Work with this weed also showed it to be an exception to the usual rule "that feed flavors are more pronounced in cream than in the milk from which the cream is taken," the flavor produced by bitterweed being more pronounced in skim milk than in whole milk and much less pronounced in the cream than in the skim milk. It further showed that there also may be exceptions to the rule that "feed flavors are not imparted to milk except for a few hours after feeding." When cows consume 10 pounds of bitterweed the flavor is present in the milk produced 24 hours later, but milk produced 27 hours later is practically free from a bitter flavor.

Babcock (21) summarizes his work by stating: "Proper methods of feeding are essential to the production of palatable milk. In most cases feed flavors are not imparted to milk except for a few hours after feeding. For this reason dairy cows should be given highly flavored feeds immediately after milking, never just before. When consumed in large quantities, feeds such as cabbage, which has an unusually strong flavor and odor, occasionally affect the quality of milk for 12 hours after feeding; but the intensity of the flavor has usually decreased to such an extent that it would not be noticed by the average consumer." He further states: "Proper aeration and cooling of strong feed flavors and odors and sometimes eliminate slight flavors

and odors. Therefore, when the practice of feeding immediately after milking is followed by proper aeration of the milk, most highly flavored feeds will not make the milk unpalatable."

Some of the feeds studied by Babcock (14-18) are mentioned in earlier literature. Vandenheydonck (22) reports a case in which the cause of a bitter flavor in milk was located in the feeding of Swedish turnips which had been washed in foul ditch water. Dammann (23) says "Bitterness in milk is often due to feedstuffs such as oat straw, turnip roots, cabbage, rapeseed cake, wormwood." Dean (24) reports feeding cows 3 pecks (41 pounds) of turnips per day with the result that a slight taint was noted in the milk. When 4 pecks (55 pounds) were fed, the milk had a decided taste of turnips. Pasteurization and added starter prevented this taste from being carried to the butter. He also reported that the flavor of butter was slightly better from mixed feed than from silage feed. Lindsey, Holland, and Smith (25) report that the feeding of dried distillers' grains or brewers' dried grain in quantities of from 3 to 4 pounds per cow per day did not affect the flavor of the milk, and regarding the feeding of beet pulp Reece (26) reports that the milk showed no uncommon flavor of any kind when 10 pounds per head per day of the best pulp slices were being fed to cows at 4 important English agricultural colleges.

The results obtained by Gamble and Kelly (2) and by Babcock (14-21) have been confirmed and extended by other investigators. Davies (27) reported that the feeding of dried beet pulp sometimes causes a fishy or off-flavor in milk. Hening and Dahlberg (28) found no abnormal flavors due to feeding mangels or dried beet pulp and at the same time concluded that these feeds in no way prevented or increased the susceptibility of milk to the development of oxidized flavor.

Roadhouse and Henderson (29) state that "full rations of alfalfa hay, green alfalfa, clover hay, or corn silage fed 1 to 2 hours before milking produced strong, undesirable feed flavors and odors. As the interval between feeding and milking increased, the intensity of the feed flavors decreased. When these feeds were withheld during the 5-hour interval before milking, objectionable feed flavors and odors were eliminated." These authors also found that when green barley, wild oats, foxtail, and filaree were fed to cows 2 hours before milking, in quantities required for satisfactory nutrition and as a sole source of roughage, undesirable feed flavors varying from slight to strong were imparted to the milk in every instance. Tame oat hay gave only slight after-flavor in milk when 8 to 9 pounds was fed to cows 2 hours before milking. When fed in a mixture with 7 pounds of alfalfa hay, it did not modify the intensity of the alfalfa flavor. Improperly cured hay having a musty odor transmitted a musty flavor to milk.

Studying the concentrates, these same investigators state "The usual concentrate feeds—rolled barley, coconut meal, soybean meal, cottonseed

meal, wheat bran, and dried beet pulp—when fed 1 or 2 hours before milking, in quantities used by the average commercial dairyman, did not give milk sufficient flavor to make it undesirable to the average consumer. Rolled barley and beet pulp, however, fed alone in 5-pound quantities or more, 1 and 2 hours before milking, gave either a detectable flavor or after-flavor; but the judges believed that these would not be noticed in cold milk by the average consumer. Wheat bran seemed to improve the flavor of the milk when fed in 5½ to 7 pound quantities 1 hour before milking. It gave more flavor to the milk than was present in the control samples, and the flavor was reported as pleasing."

In a study to determine the rate at which the juice of the alfalfa plant made its appearance as a feed flavor in the milk, Roadhouse and Henderson (30) concluded: "Feed flavor appears in milk 20 minutes after the ingestion of flavor-producing materials in liquid form. The most pronounced feed flavor was present in the milk drawn 45 to 60 minutes after drenching."

These same investigators (Roadhouse and Henderson) (31) found that when cows were fed alfalfa hay as roughage immediately after milking or were given access to alfalfa pasture both day and night for the entire interval between milkings, they did not consume sufficient feed during the last 5 hours before milking to seriously affect the flavor of the milk. When they were given access to the pasture only, during the interval between morning and evening milkings, they consumed sufficient feed during the 5-hour interval before milking to cause an objectionable feed flavor in the milk. Under these conditions they recommend that the cows be removed from the pasture 4 or 5 hours before milking if feed flavor in milk is to be entirely avoided. They also recommend that if large amounts of corn silage are used in the ration it be fed after milking.

Lucas (32) stated "Alfalfa hay gives to milk a rather pronounced flavor but it is objected to only by a very few people." Weaver, Kuhlman, and Fouts (33) concluded from their work with alfalfa hay that: "Alfalfa hay fed less than four hours before milking has a pronounced effect on milk flavor. This effect is observed even when the interval between feeding and milking is only one-half hour. The two-hour interval causes the most serious flavor in the milk. If the hay is fed as long as four hours before milking the flavor is entirely eliminated with some cows. With other cows it is so reduced as to be scarcely discernible. Aeration of the milk removes some of the flavor but does not entirely eliminate it. Cooling seems to be ineffective and the effect of alfalfa hay is far more serious than that of darso silage."

The important part which feeds play in the flavor of milk has been further exemplified by Weaver, Fouts, and McGilliard (34). These investigators have shown that feed flavors are the most prevalent of the numerous flavor defects encountered in milk.

## SUMMARY

The investigators on the effect of feeds on the flavor and odor of milk have shown that:

Many feeds impart their flavor to milk, the intensity of the imparted flavor depending upon the character of the feed, quantity consumed, and the time the feed is consumed in relation to the time of milking.

Feed flavors enter milk mainly through the body of the cow and in most cases these flavors are not imparted to milk except for a few hours after feeding.

Strong feed flavors are reduced in intensity and slight flavors may be eliminated by proper aeration.

Highly flavored feeds should be fed immediately after, never just before, milking.

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# American Dairy Science Association Announcements

## HUBERT EVERETT VAN NORMAN

JANUARY 30, 1872—JULY 28, 1938

Hubert Everett VanNorman, a professor of Dairy Husbandry and a prominent executive in educational and industrial institutions, died in Chicago, Illinois, on July 28, 1938.

Few men who have started their careers in the classroom and held prominent executive positions in educational institutions have been able to devote as much time to leadership in the dairy industry. He was reared on a dairy farm and with that background he had the interests of the dairy farmer at heart. During the years he was connected with educational work, he would turn aside his collegiate duties at any time to speak to dairy gatherings or attend dairy industry meetings in which he always took a prominent part. He was a clear, inspiring speaker. This quality and his broad experience in the dairy industry led to the continual demand for his services by dairy organizations. He once remarked that his greatest ability seemed to be as a speaker and a starter of new enterprises.

Dr. VanNorman was born at Tillsonburg, Canada, and came to the United States in 1880. He was graduated from the Michigan Agricultural College in 1897 and served as assistant buttermaker during his college course. After graduation he accepted a position at Purdue University as farm superintendent and instructor in dairying and was advanced to Chief of the dairy department in 1902, which position he held for four years. While at Purdue, he served for seven years as Secretary of the Indiana Dairy Association. In 1905 he became Professor of Dairy Husbandry at the Pennsylvania State College and was Head of the Department until 1913 when he resigned to accept a position with the University of California as Professor of Dairy Management, Vice-director of the Agricultural Experiment Station and Dean of the University Farm School.

While at Pennsylvania State College, Dr. VanNorman was active in improving the College instruction and was the author of the text book "First Lessons in Dairying." During these years he took a prominent part in state and national dairy association affairs and served as President of the Pennsylvania Dairy Union, secretary of the Agricultural Federation of Pennsylvania and Vice-President of the National Dairy Show in 1907-8. He was secretary and manager of the National Dairy Show for two years and finally president in 1911, which honor he held until 1922. While president of the National Dairy Show, Dr. VanNorman contributed much to the progress of dairying and the dairy industry. His speaking ability and dignity on the speaking platform brought him before many of the industry conventions and he held the dairy cattle equipment and machinery organizations together for

many years in a unified Dairy Exposition. During these years he served also as a director of the National Dairy Council and he was a charter member of the American Dairy Science Association. Although Dean VanNorman's duties were arduous in California in developing a recently established school of agriculture, he found time to attend meetings of the directors of the National Dairy Show and to spend a week at the Show to fulfill his responsibilities as president.

In 1921 plans were being made in Washington to invite the World's Dairy Congress to meet in the United States. Dean VanNorman was asked to serve as organizer and manager of the Congress. This important undertaking required the services of a man of ability in leadership who was well known to the dairy industry, and Dr. VanNorman was a happy selection for this important position. To assure representation of European countries at the Congress, Dr. VanNorman visited many countries in Europe and extended personal invitations. The Congress which was held in Washington, Philadelphia, and Syracuse in 1923, was splendidly organized and carried out. At the close of the Congress at Syracuse, the University of Syracuse conferred upon Dean VanNorman the honorary degree of Doctor of Laws.

In 1925 he was selected to organize the American Dry Milk Institute and he became its first president. In 1929 he resigned to become director of research for the Borden Company, which post he in 1903 left to take charge of the dairy industry exhibit at the Century of Progress Exposition in Chicago. At the close of the exposition Dr. VanNorman was Director of Development and Education for the Chicago Mercantile Exchange.

Those who knew Dr. VanNorman as a close personal friend, and who had been a guest in his home, realized his cordial and lovable nature.

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## EFFECT OF SHAKING ON THE LIPOLYSIS OF COW'S MILK<sup>1</sup>

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### INTRODUCTION

Eufinger (10) showed that the titratable acidity of human milk increased several fold as a result of shaking for a few hours, and that the increase was associated with the presence of fat since the acidity did not increase when skimmilk was shaken. He found further that the increase in acidity was slight when cow's milk was shaken, and concluded that this difference could be used to distinguish between cow's and human milk. Engel (8, 9) was loath to accept the idea that the increase was the result of lipase action, because usually enzymes are inactivated by shaking. Davidsohn (5), Behrendt (2, 3), Schlossman (19), and Freudenberg (11) concluded that shaking activated the enzyme or cleared the surface of the fat globules for action. A difference in the effect of shaking on the increase in acidity of cow's and human milk was noted by Eufinger (10), Davidsohn (5) and Behrendt (2), and the two last named investigators observed a marked alteration in surface tension as a result of shaking human milk. Krukovsky and Sharp (14) found a marked increase in titratable acidity due to lipolysis during the churning of raw cream separated from the milk of certain cows in advanced lactation. This result indicated that the lipase of cow's milk could be activated by shaking, and that shaking could be used as a method for activating and studying the true lipase of milk. Some of the results obtained are presented in this paper.

Paraschtschuck (18), Eckles and Shaw (7), Palmer (17), Sharp and de Tomasi (20), Csiszár (4), Hileman and Courtney (13), Anderson (1), Krukovsky and Sharp (14) and others have shown the marked lipolytic activity of milk from certain cows, particularly those in advanced lactation.

Most of these investigators are of the opinion that some other factor in addition to advanced lactation is necessary for the production of milk in which the lipase is naturally active. This contributing factor may be temperature, season, feed, or some other factor affecting the physiology of milk secretion or its composition.

<sup>\*</sup> Received for publication May 4, 1938.

<sup>1</sup> We are indebted to the Joseph Willmann Dairy Research Fund for a grant in aid of this investigation.

## EXPERIMENTAL

The method consisted in the determination of the increase in titratable acidity expressed as percentage lactic acid and decrease in pH which resulted from shaking 25 ml. of milk in 75 ml. test tubes. The tubes were shaken violently in a motor-driven machine which was placed in a room of constant temperature.

Table 1 shows that samples of raw milk from different cows decreased an average of 0.30 pH when shaken for 2 hours at 37° C.; whereas pasteurized aliquots shaken, raw milk held for 2 hours at 37° C. without shaking, or held over night at 5° C., showed no change in pH.

TABLE 1

*Effect of 2 hours' shaking at 37° C. on the pH of raw and pasteurized whole milk*

Sample number	Fresh raw pH	Over night at 5° C. raw pH	2 hours at 37° C.			
			Raw held unshaken pH	Shaken 2 hours		
				Past. pH	Raw pH	Dif. pH
1	6.52	6.53	6.50	6.52	6.22	0.30
2	6.64	6.66	6.64	6.66	6.41	0.25
3	6.63	6.66	6.62	6.63	6.35	0.28
4	6.53	6.55	6.48	6.55	6.16	0.39
5	6.55	6.56	6.54	6.55	6.27	0.28
6	6.62	6.63	6.60	6.62	6.28	0.34
7	6.61	6.52	6.42	6.52	6.22	0.30
8	6.65	6.56	6.51	6.60	6.23	0.37
9	6.49	6.52	6.50	6.49	6.35	0.14
10	6.56	6.60	6.57	6.57	6.22	0.35
Average ...	6.58	6.58	6.54	6.57	6.27	0.30

Experiments of a similar type in which the milk was shaken for 2 hours at 25° C. with and without the addition of ethyl butyrate are presented in Table 2. The first group of samples were from cows in the middle of the lactation period, the second group from cows at the end of the lactation period. The experiments were run during November and December of 1935. The samples from the group of cows in the more advanced period of lactation did not show greater lipolytic activity as a result of shaking. The increase in acidity as a result of shaking was slightly greater in the presence of ethyl butyrate, which was added originally because it usually prevents churning. The increases in titratable acidity parallel the decreases in pH. The acidity increased less with shaking at 25° C. than at 37° C.

TABLE 2

*Effect of 2 hours' shaking at 25° C. on the increase in acidity of raw and pasteurized milk*

*Sample Nos. 1 to 10 from cows relatively high in milk production*

*Sample Nos. 11 to 20 from cows relatively low in milk production*

Sample number	pH						Acidity as % lactic acid								
	Natural whole milk			Natural whole milk + 2% ethyl but.			Natural whole milk			Natural whole milk + 2% ethyl but.					
	Raw	Past.	Dif.	Raw	Past.	Dif.	Raw	Past.	Dif.	Raw	Past.	Dif.	Raw	Past.	Dif.
	<i>pH</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>	<i>pH</i>	%	%	%	%	%	%	%	%	%
1	6.25	6.54	0.29	6.18	6.57	0.39	.227	.170	.057	.245	.166	.079			
2	6.29	6.54	0.25	6.17	6.54	0.37	.220	.175	.045	.230	.175	.055			
3	6.28	6.47	0.19	6.21	6.45	0.24	.225	.185	.040	.235	.187	.048			
4	6.40	6.64	0.24	6.22	6.62	0.40	.172	.120	.052	.202	.125	.077			
5	6.21	6.62	0.41	6.13	6.61	0.48	.260	.160	.100	.270	.155	.115			
6	6.20	6.49	0.29	6.11	6.49	0.38	.262	.185	.077	.292	.187	.105			
7	6.32	6.43	0.11	6.26	6.43	0.17	.217	.190	.027	.222	.190	.032			
8	6.26	6.53	0.27	6.08	6.49	0.41	.235	.175	.060	.265	.180	.085			
9	6.25	6.41	0.16	5.99	6.40	0.41	.225	.197	.028	.250	.195	.055			
10	6.37	6.53	0.16	6.20	6.53	0.33	.220	.175	.045	.240	.175	.065			
Average	6.28	6.52	0.24	6.16	6.51	0.36	.226	.173	.053	.245	.174	.071			
11	6.32	6.62	0.30	6.28	6.58	0.30	.190	.132	.058	.195	.137	.058			
12	6.47	6.66	0.19	6.40	6.65	0.25	.195	.155	.040	.205	.155	.050			
13	6.33	6.65	0.32	6.26	6.64	0.38	.185	.125	.060	.200	.130	.070			
14	6.45	6.82	0.37	6.40	6.82	0.42	.180	.110	.070	.190	.110	.080			
15	6.62	6.80	0.18	6.60	6.80	0.20	.175	.130	.045	.175	.130	.045			
16	6.36	6.63	0.27	6.26	6.63	0.37	.200	.125	.075	.227	.125	.102			
17	6.51	6.68	0.17	6.56	6.72	0.16	.180	.137	.043	.180	.137	.043			
18	6.38	6.67	0.29	6.31	6.67	0.36	.197	.130	.067	.210	.132	.078			
19	6.58	6.81	0.23	6.58	6.82	0.24	.157	.117	.040	.160	.120	.040			
20	6.29	6.52	0.23	6.26	6.50	0.24	.225	.165	.060	.240	.170	.070			
Average	6.43	6.68	0.25	6.39	6.68	0.29	.188	.133	.055	.198	.134	.064			

Table 3 shows that the acidity increases with time of shaking, the greatest increase occurring in the first two hours. After a preliminary shaking at 25° C., the acidity continues to increase even when the milk is held afterward at 2° C. The relative increases are much the same when 2 per cent tributyrin is added, but the actual increases in acidity are greater, and the unshaken samples containing tributyrin show a marked increase in acidity on holding. Table 3 shows that milk which normally shows no lipolytic activity is capable of activation to produce definite lipolysis.

Temperature is a controlling factor in lipolytic activation of milk by shaking. If the milk is held and shaken at low temperatures the lipase is not activated. Table 4 shows a comparison of milk shaken at 2° and at 25° C. Definite lipolysis of natural fat occurred in the case of only one sample which was shaken and held at 2° C. This sample was undoubtedly one of those in which the lipase was naturally active. Since shaking is not

TABLE 3

*Effect of time of shaking at 25° C. and time of subsequent holding at 2° C. on the increase in titratable acidity and decrease in pH of raw milk*

Time of holding at 2° C. after shaking, hours	Control	Natural raw whole milk						Raw whole milk + 2% tributyrin					
		Hours of shaking at 25° C. prior to holding at 2° C.						Hours of shaking at 25° C. prior to holding at 2° C.					
		0	½	1	2	3	4	0	½	1	2	3	4
		Increase in titratable acidity, per cent											
0	.001	.005	.013	.022	.038	.049	.055	.017	.037	.041	.078	.097	.106
24	.001	.018	.042	.049	.069	.067	.085	.063	.132	.126	.147	.154	.161
48	.001	.029	.059	.069	.084	.089	.094	.105	.174	.171	.189	.175	.187
72	.005	.041	.075	.082	.092	.102	.109	.125	.204	.206	.222	.209	.219
Decrease in pH													
0	.02	.00	.07	.10	.16	.17	.21	.14	.42	.42	.57	.61	.67
24	.07	.06	.19	.20	.29	.30	.32	.43	.71	.67	.77	.75	.77
48	.05	.11	.23	.26	.32	.32	.36	.56	.78	.84	.86	.87	.88
72	.03	.19	.27	.32	.35	.41	.41	.63	.88	.87	.96	.93	.93

necessary to induce the hydrolysis by raw milk of simple esters such as tributyrin and ethyl butyrate, the samples to which the esters were added and which were shaken and held at 2° C. showed appreciable hydrolysis when held for 24 hours.

Most of the experiments on lipolysis due to shaking, previously reported in the literature, had been performed with human milk. Table 5 shows that little lipolysis of human milk occurs as a result of shaking at a low

TABLE 4

*Effect of holding for 24 hours at 2° C. on the increase in titratable acidity after shaking for 2 hours at 25° and 2° C. Milk from cows late in lactation*

	Cow number				
	1	2	3	4	5
Direct titratable chloride value, per cent	.137	.129	.178	.143	.152
Titratable acidity, unshaken sample, per cent	.175	.160	.092	.165	.140
pH	6.59	6.52	6.86	6.52	6.64
Increase in titratable acidity					
Natural raw milk shaken 2 hrs. at 25° C.	.055	.030	.035	.047	.047
Shaken 2 hrs. at 25° C. and held 24 hrs. at 2° C.	.090	.058	.052	.072	.078
Raw milk + 2% ethyl butyrate shaken 2 hrs. at 25° C.	.055	.060	.040	.053	.063
Shaken 2 hrs. at 25° C. and held 24 hrs. at 2° C.	.113	.110	.070	.105	.125
Natural raw milk shaken 2 hrs. at 2° C.	.000	.000	.010	.002	.022
Shaken 2 hrs. at 2° C. and held 24 hrs. at 2° C.	.000	.018	.007	.010	.038
Raw milk + 2% ethyl butyrate shaken 2 hrs. at 2° C.	.020	.015	.015	.010	.020
Shaken 2 hrs. at 2° C. and held 24 hrs. at 2° C.	.100	.105	.050	.080	.085

pH determinations were also made. They confirm the results of the titratable acidity values.

TABLE 5

*Effect of temperature of shaking on the lipolysis of human milk as indicated by the increase in titratable acidity expressed as lactic acid*

Time of experiment, 1 hour	Temperature	
	5-6° C. Titratable acidity per cent	25-28° C. Titratable acidity per cent
Unshaken	.023	.023
Shaken	.027	.108
Shaken	.032	.108

temperature, as contrasted with shaking at 25° C. These results confirm previous investigations indicating that lipolysis of human milk is stimulated to a greater extent by shaking than is lipolysis of cow's milk.

Anderson (1) and Mattick and Kay (16) used tributyrin as a substrate for studying the lipolytic activity of cow's milk. Experiments indicate that shaking is not necessary to induce hydrolysis of simple esters. The fact that no activation is necessary to cause the hydrolysis of simple esters, whereas activation is necessary to induce the true lipolysis of the natural fat, serves to differentiate the two reactions. The hydrolysis caused by

TABLE 6

*Effect of the combination of ethyl butyrate and tributyrin on the increase in titratable acidity and decrease in pH. Shaken (2 hours at 25° C.) and unshaken milk after holding at 2° C. for 24 hours*

	Increase in titratable acidity		Decrease in pH	
	Shaken %	Unshaken %	Shaken pH	Unshaken pH
Sample No. 1				
0.118% apparent titratable Cl				
Natural raw milk	.095	.005	.34	.00
Raw milk + 2% ethyl-n-butyrate	.142	.115	.64	.51
Raw milk + 2% tributyrin	.168	.127	.88	.67
Raw milk + { 1% ethyl-n-butyrate	.142	.122	.75	.64
Raw milk + { 1% tributyrin				
Raw milk + { 2% ethyl-n-butyrate	.155	.137	.81	.73
Raw milk + { 2% tributyrin				
Sample No. 2				
0.178% apparent titratable Cl				
Natural raw milk	.050	.007	.32	.05
Raw milk + 2% ethyl-n-butyrate	.065	.067	.36	.36
Raw milk + 2% tributyrin	.100	.070	.62	.49
Raw milk + { 1% ethyl-n-butyrate	.095	.073	.67	.54
Raw milk + { 1% tributyrin				
Raw milk + { 2% ethyl-n-butyrate	.095	.075	.67	.54
Raw milk + { 2% tributyrin				

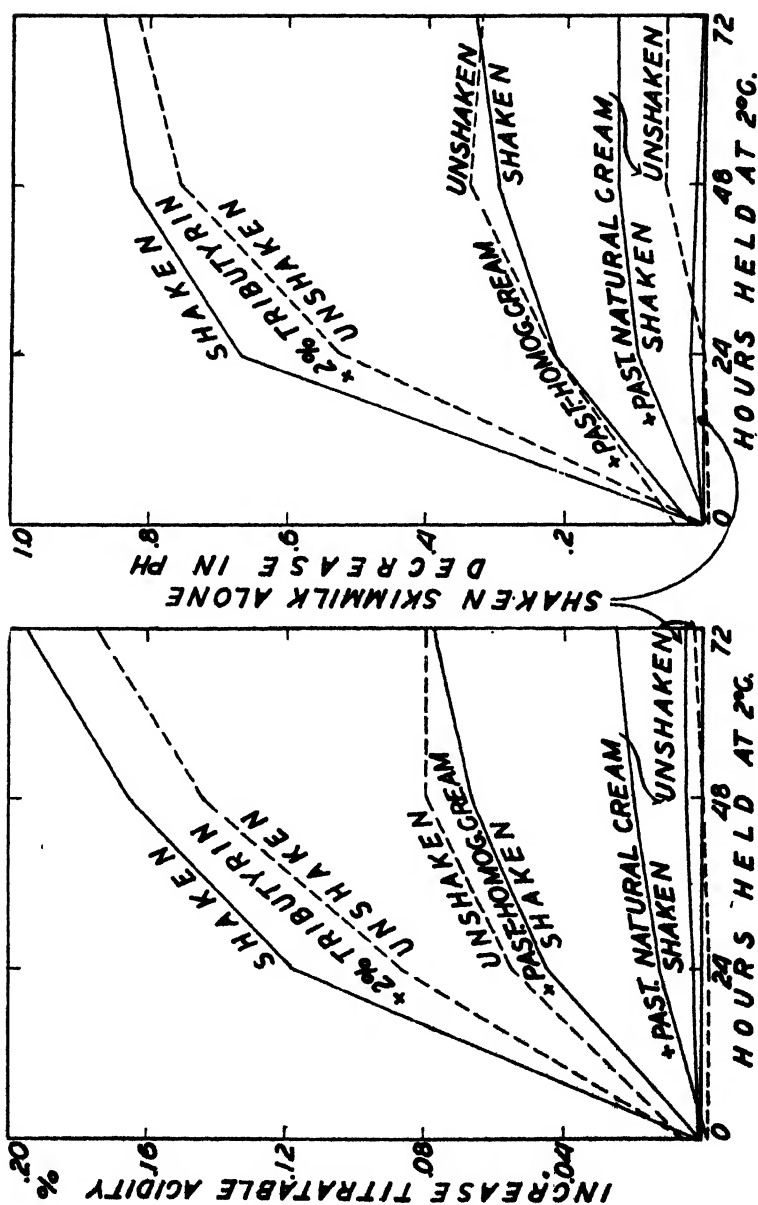


Fig. 1. Effect on lipolytic activity of reconstituting shaken and unshaken skimmilk with homogenized and natural cream and the addition of tributyrin.

shaking milk to which the simple esters were added was greater than that of the milk when shaken alone, but the increase in acidity was not the sum of the two processes operating alone. Table 6 presents further evidence that the increases in acidity produced by shaking milk alone and when both tributyrin and ethyl butyrate are present is not additive. The increase in acidity seems to approach a limiting value, indicating the establishment of an equilibrium or the retardation of the action of the enzyme due to the low pH.

As indicated in Figure 1, little subsequent effect of the shaking was found when 2% tributyrin, 4% homogenized cream, or 4% natural cream was added to shaken and unshaken skimmilk. Marked lipolysis occurred when pasteurized homogenized cream was added to raw skimmilk, but not when pasteurized natural cream was added.

No important influence of breed on the increase in acidity as a result of shaking was found, as shown by Table 7, although the milk from Jersey

TABLE 7

*Effect of breed on the increase in acidity produced by shaking for 2 hours at 25° C.*

	Decrease in pH		Increase in titratable acidity	
	Natural milk	Natural milk + 2% ethyl butyrate	Natural milk	Natural milk + 2% ethyl butyrate
	<i>pH</i>	<i>pH</i>	%	%
Holstein	.23	.31	.049	.059
Jersey	.25	.31	.067	.076
Guernsey	.23	.33	.048	.061
Ayrshire	.23	.30	.052	.064
Average	.24	.31	.054	.065

cows showed slightly greater increases in acidity.

A definite relation exists between the increase in titratable acidity and the decrease in pH, as shown in Figure 2, but since with the same increase in titratable acidity different alterations in pH would occur because of the difference in the buffer value of the milk, the increase in titratable acidity would be more directly related to the lipase activity.

The increase in titratable acidity as a result of shaking the natural milk for 2 hours at 25° C., although less, is in general linearly related to the increase if ethyl butyrate or tributyrin is added, as shown in Figure 3.

No relation between the amount of milk produced per day and the increase in titratable acidity resulting from shaking is shown in Figure 4.

The milk from a large number of cows was tested to see if the lipase was naturally active. The milk was cooled at once and held cold for 2 days.

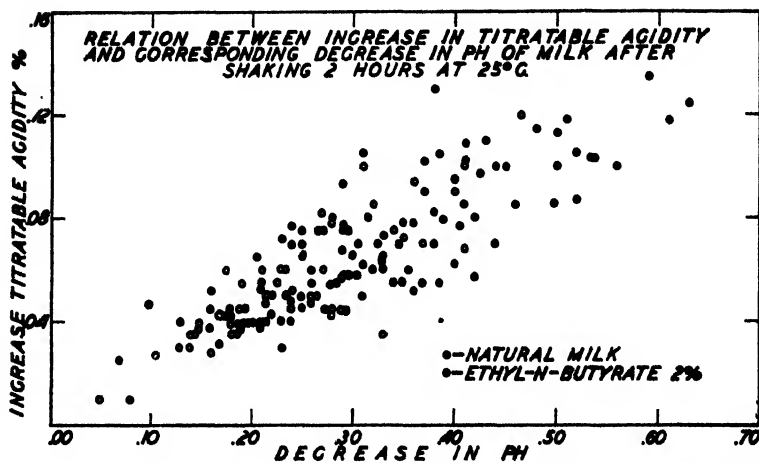


FIG. 2. Relation between the increase in titratable acidity expressed as lactic acid and the decrease in pH as a result of shaking.

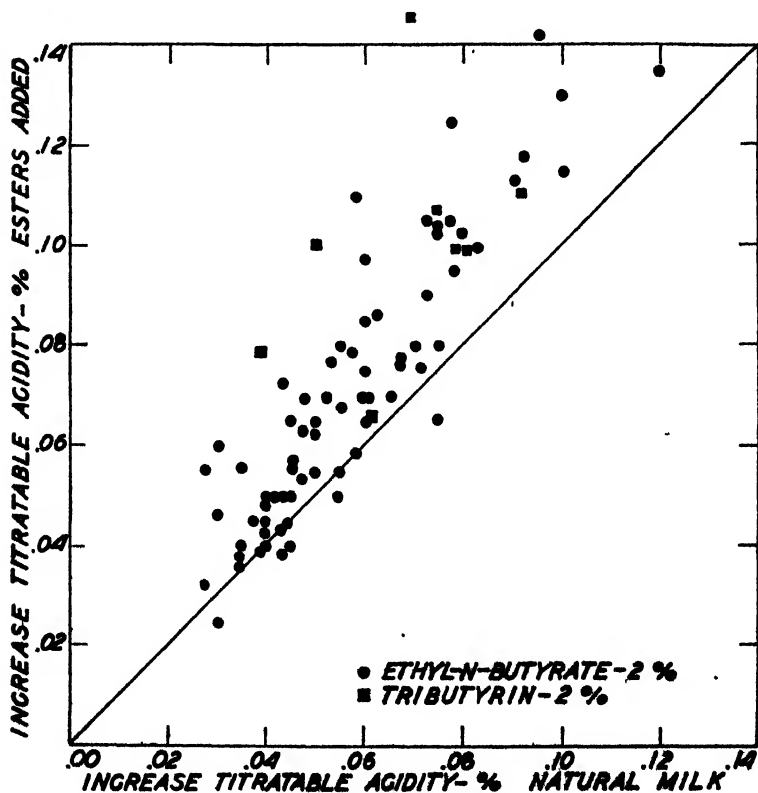


FIG. 3. Effect of the addition of esters on the increase in titratable acidity produced by shaking milk for 2 hours at 25°C.

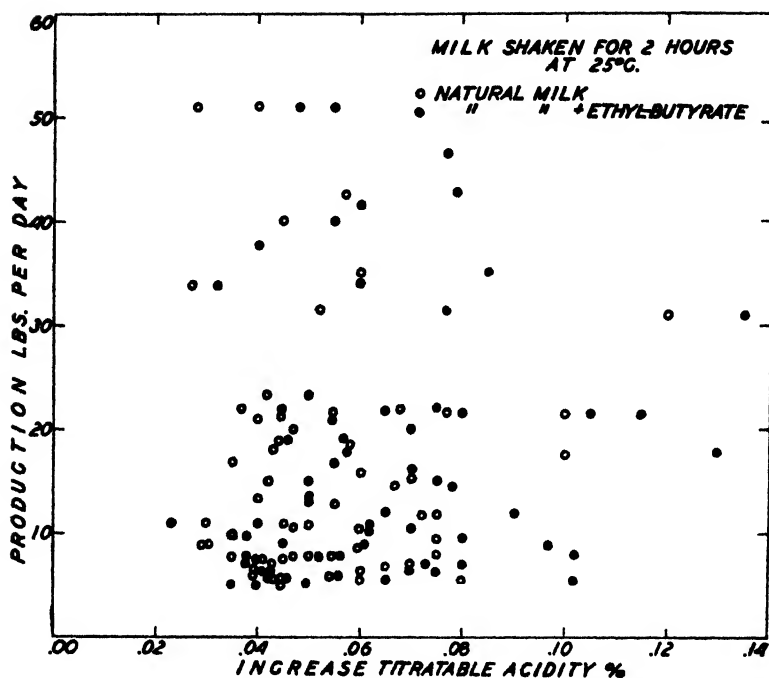


FIG. 4. Relation between the production of milk in pounds per day and the increase in titratable acidity produced by shaking.

An increase in titratable acidity on holding was taken as an indication that the lipase was naturally active. The milk from a large number of cows in advanced lactation in the summer was tested, but none showed an increase in titratable acidity. A few cows in advanced lactation in winter

TABLE 8

*Milk obtained in the winter which showed natural lipolytic activity*

Cow No.	Increase in titratable acidity as a result of		Direct titratable chloride value	Month of lactation	Milk per day
	Holding unshaken at 2° C. for 48 hrs.	Shaking 2 hrs. at 25° C.			
	%	%	%		lbs.
1 Jersey .....	0.020	0.053	0.09	10	7.7
2 Jersey .....	0.024	0.055	0.12	11	7.0
3 Holstein .....	0.028	0.025	0.15	11	9.0
4 Holstein .....	0.055	0.055	0.15	12	2.2
5 Holstein .....	0.038*	0.047	0.15	..	10.6

\* Held cold 24 hours.

gave milk in which the acidity increased on holding. The data are presented in Table 8. Although this milk was naturally lipolytically active, it showed about the same lipolysis as a result of shaking as did average normal milk.

#### DISCUSSION

Shaking raw milk while the fat is in the liquid or partially liquefied state activates the lipase naturally present. Hydrolysis of the fat is indicated by the increase in titratable acidity, decrease in pH, and the characteristic odor and taste of the milk. Although shaking apparently gives no indication as to whether the lipase is naturally active in the milk, it does afford a simple method of demonstrating quickly the lipolytic potentialities of the milk.

Dorner and Widmer (6) found that the lipase of raw milk could be activated by homogenization. This fact has been confirmed by others (20), and Gould and Trout (12) showed that tremendous increases in the acidity of the fat occurred. Van Dam (21) has shown that the degree of dispersion of the fat is increased by shaking milk while the fat is in the liquid state. We have confirmed this observation. Thus activation of lipase by homogenization and by shaking may be basically the same. Whether an increase in degree of dispersion is necessary in order to activate the lipase of the milk by shaking, or whether the activating mechanism is an alteration in the surface characteristics of the fat globules, is not clearly demonstrated by the shaking experiments alone. The alteration in the surface characteristics is believed to be the activating factor, the increase in the degree of dispersion being largely incidental. This belief is strengthened by the known influence of temperature of separation on the lipolytic activity of cream (20). Cream from milk, warmed and separated while the fat is in the liquid state, shows little lipolytic activity as contrasted to cream separated while the fat is in the solid or partially solid state. Both creams show about the same amount of lipolytic activity when subsequently activated by homogenization. This indicates that lipase is actually present in both creams, but because of the previous temperature history of the fat and its physical state, in one case the lipase is active, in the other not.

Lundstedt (15) claimed that whipping cold milk lowered the curd tension of the milk. He attributed this lowering of the curd tension to materials removed from the surface of the fat globules as a result of the agitation. In our experiments, shaking cold raw milk did not induce lipolysis of cow's milk nor of human milk.

Under certain conditions raw milk from some cows in advanced lactation naturally shows lipolysis when held cold for a day or two. It is but natural to draw the conclusion that such milk contains more lipase than does other more normal milk which does not increase in acidity under the same conditions. However, when both types of milk are subjected to

special activating treatments such as to shaking or homogenization, they both show about the same amount of lipolytic activity. These results point to the conclusion that, although the amount of lipase as evidenced by shaking may vary considerably, yet all milk contains enough lipase which if activated would produce rancid milk, but only in the case of certain cows, usually in advanced lactation, is the condition of the milk such that when cooled at once the lipase is in a naturally activated state. We use the term activation in a broad sense to include the possibility that the apparent activation may be due to the prevention of the action of an inactivator.

#### CONCLUSIONS

1. Shaking of raw, whole cow's milk, while the fat is in the liquid or partially liquefied state, induces lipolysis.
2. Lipolysis induced by shaking will continue after the milk has been cooled to low temperatures.
3. The amount of lipolysis induced by shaking bears little or no relation to breed, season, or milk production of the cow.
4. The effect of shaking is attributed to an alteration in the surface characteristics of the fat globules which creates a condition more favorable for lipolysis.
5. Apparently all milk is capable of appreciable true lipolytic activity if subjected to suitable activating treatments, but only from some cows, particularly those in advanced lactation in winter, is milk secreted which when cooled and held will show natural lipolytic activity.

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# VOLUME CHANGES OF FAT IN COOLED CREAM HELD AT CONSTANT TEMPERATURE

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## INTRODUCTION

The increase in the specific gravity of milk when held cold at a constant temperature has been observed by Schröder (4), Toyonaga (6), and many others (2). Toyonaga explained the increase as due to the solidification of the fat. Attention has recently been called to the influence of the physical state of the fat on the specific gravity determination (5). The evidence is clear that a shrinkage in volume occurs at constant temperature when milk or cream is cooled quickly and held at temperatures which induce fat crystallization. Because of the much greater lag in phase change of fat globules, the time required for fat in mass to crystallize gives little information as to the time required for fat globules in milk or cream to crystallize. A study of the change in physical state of the fat as indicated by specific heat determinations indicated that about 4 hours were required for the physical state to approximate the equilibrium value (3). In the present study, dilatometers were used to follow the change in physical state of the fat at constant temperature.

The dilatometer has previously been used principally to measure the expansion of the fat in cream, on warming after having been subjected to different degrees of cooling. Van Dam (8, 9) found that the maximum expansion occurred between 12 and 18° C. Hansen and Jensen (1) also showed that the more the cream was cooled the greater the expansion of the fat on warming.

## EXPERIMENTAL

The construction of the dilatometer used is illustrated in Figure 1. The bore of the stopcock was large enough to permit the insertion of a drawn out glass tube through which the cream was introduced. This permitted the escape of the air and the complete filling of the bulb. The bulbs held 35 to 40 ml. The distance between the level of the mercury in the side arm capillary and a reference mark on the capillary was determined by means of a cathetometer. The diameter of the capillary side arm was previously determined by measuring the length of a mercury thread and weighing the mercury. From the movement of the mercury due to contraction of the fat, the contraction in volume per 100 grams of fat was calculated.

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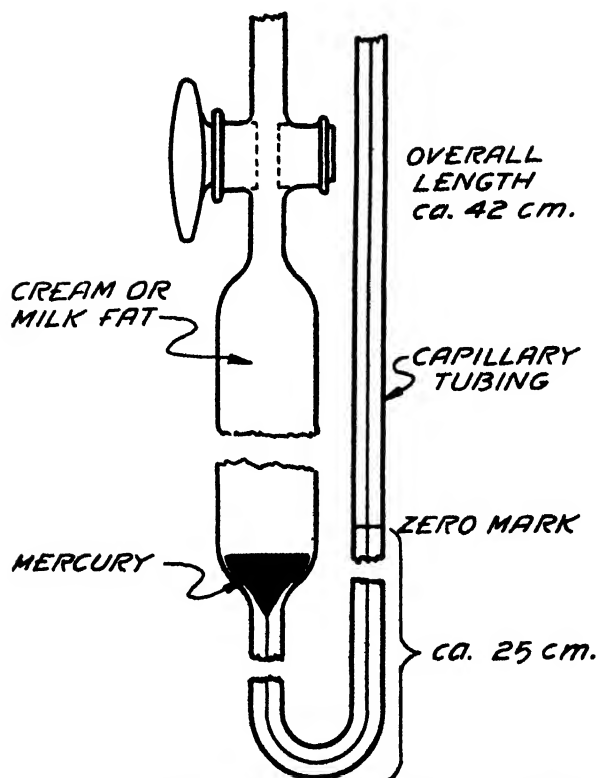


FIG. 1. Dilatometer used to measure the change of volume of fat in cream.

Several hours before beginning the experiment, the weighed dilatometers were placed in water baths maintained at the desired temperature by being held in thermostatically controlled air ovens which in turn were placed in refrigerated rooms. Cream from mixed milk was heated to  $80^{\circ}\text{C}$ . for 10 minutes, and cooled to  $40^{\circ}\text{C}$ ., when 0.5 per cent of mercuric chloride was added (7). The cream was then filtered through cotton and stored at near freezing. Before being placed in the dilatometer the cream was warmed to  $45^{\circ}\text{C}$ . to melt the fat in the globules. It was then cooled to the temperature of the water bath, and the dilatometer was filled.

The dilatometers were held for five months at constant temperatures ranging from 0 to  $20^{\circ}\text{C}$ . The contraction in cc. per 100 grams of fat during the first 48 hours is plotted in Figure 2. The decrease in volume of the fat occurred rapidly at 0, 5, and  $10^{\circ}\text{C}$ . after adjusting the cream to the temperature of the bath; the contraction took place more slowly at  $15^{\circ}\text{C}$ . and very slowly at  $20^{\circ}\text{C}$ . Some contraction occurred at the lower temperatures before the cream could be cooled, adjusted to the temperature of the bath, the tonometer filled and the first reading taken. Therefore the data presented in Figure 2 should not be taken as an accurate indication

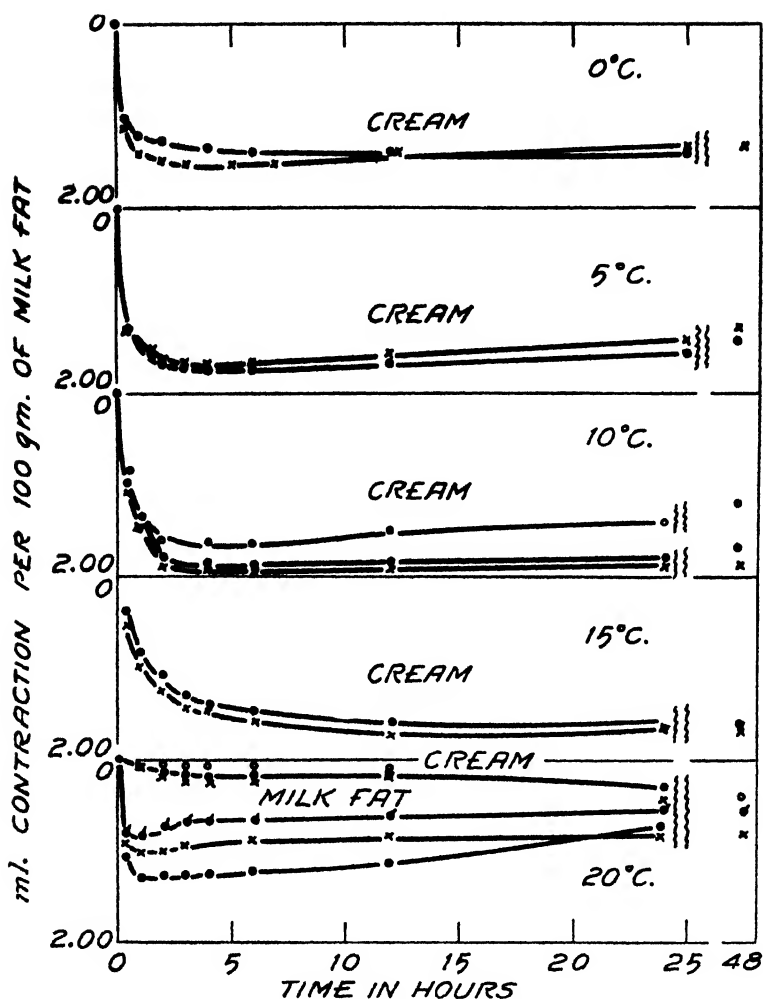


FIG. 2. Change in volume of milk fat in cream and in mass when held at constant temperature. Calculated on the basis of cc. change in volume per 100 grams of fat.

of the total contraction which occurred at the lower temperatures. The curves indicate the rate of contraction as influenced by temperature. At temperatures below 10° C., after the very rapid decrease in volume a slow increase in volume occurred. The increase occurred over a period of months, as is shown more clearly in Table I. At the present time the explanation for this slow expansion is not clear, but it is probably due to some slow phase adjustment following the relatively rapid original crystallization of the fat. The secondary expansion was not observed in cream held at 15 and 20° C., but it was observed in pure fat held at 20° C. The decrease in volume of the fat in cream held at 25° C. occurred so slowly

TABLE I

*Decrease in volume of fat on holding cream and pure milk fat for various periods of time at constant temperature*

Temperature of holding	cc. decrease in volume per 100 grams of milk fat after							
	30 min.	1 hour	4 hrs.	1 day	1 week	1 mo.	2 mo.	5 mo.
° C.	40% fat cream							
0	1.01	1.23	1.36	1.38	1.30	1.08	0.95	
	1.14	1.41	1.55	1.39	1.28	1.15	1.16	1.12
5	1.32	1.48	1.75	1.60	1.20	1.09	1.12	1.25
	1.30	1.46	1.71	1.49	1.12	.92	.81	.99
10	1.05	1.46	1.84	1.81	1.69	1.68	1.68	1.57
	1.08	1.48	1.90	1.92	1.92	1.94	1.92	2.07
	0.89	1.31	1.60	1.38	1.13	1.24	1.37	1.76
15	0.41	0.82	1.35	1.54	1.65	1.71	1.67	1.97
	0.52	0.98	1.49	1.68	1.72	1.71	1.75	2.29
	0.37	0.84	1.34	1.54	1.68	1.78	1.87	2.35
20	0.00	0.06	0.19	0.28	0.29	0.40	0.59	1.52
	0.00	0.12	0.27	0.40	0.44	0.54	0.81	1.34
	0.00	0.03	0.17	0.29	0.32	0.40	0.76	1.33
	Pure milk fat							
20	0.96	1.05	0.85	0.77	0.77	0.74	0.68	0.80
	0.85	0.80	0.67	0.63	0.59		0.41	0.31
	1.03	1.30	1.27	0.70	0.60		0.48	0.39

that dilatometers were not held at this temperature. The changes in volume of the fat in cream held for the longer periods of time are given in Table I.

Table I shows that it may require weeks or months for the fat to attain a state of equilibrium when held at constant temperatures of 20° C. or below. This table emphasizes again the unsuitability of temperatures at which the fat may be in the crystalline state, for obtaining the specific gravity values which are used for the calculation of total solids (5).

Although Figure 2 and the specific heat values previously reported (3) indicate that within 4 hours phase adjustment of the fat approaches a relatively stable state, yet Table I shows that superimposed on this apparently stable state is a secondary adjustment which may require months. In Table I the data for 10° C. are not consistent. This temperature is probably near the point at which the two behaviors are occurring simultaneously at near the same rate, that is, the primary crystallization which causes a decrease in volume and the secondary adjustment of phases which results in an increase in volume.

Observations on surface tension, creaming, cream viscosity, lipase action, foaming, etc., indicate that it is the adjustment of the surface condi-

tions of the fat globules, as produced by the crystallization which occurs in the first few hours, which is most important in controlling the behavior of milk products.

#### CONCLUSIONS

1. At 0, 5, and 10° C. the maximum contraction of the fat in cooled cream occurs in about 4 hours. At 15 and 20° C. contraction of the fat may take place over a period of months.

2. At 0 and 5° C. after the maximum contraction of the fat in cream, a slow expansion occurs for two months or more. This second stage of expansion is probably a phase adjustment following the initial rapid crystallization of the fat.

3. The adjustment of the physical state of the fat globules which at low temperature approaches completion in about 4 hours, is the important change which alters the surface properties and adsorption on the fat globules, and influences such properties as cream viscosity, creaming, surface tension, foaming, lipase, etc.

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# STUDIES ON THE COMPOSITION OF BOVINE BLOOD

## I. THE MAGNESIUM CONTENT OF THE BLOOD PLASMA OF THE NORMAL DAIRY CALF\*

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A study of some of the constituents in the blood of dairy cattle was undertaken as a phase of a more comprehensive investigation of the problem of requirements and metabolism of certain elements by dairy cattle in relation to the occurrence of deficiency diseases and with special reference to the use of home-grown feeds for economical milk production. During the past few years our attention has been directed towards a study of the magnesium requirements of cattle (1-4). In extending this investigation it was found desirable to determine the concentration and normal variations of this element in the blood plasma of growing calves over long periods of time since a review of the available literature on the magnesium content of calf blood indicated a general lack of agreement concerning the normal concentration in the blood of calves at various ages. A systematic study, therefore, has been made in which the magnesium, calcium, inorganic phosphorus, chloride and carbon dioxide content has been repeatedly determined from birth to 18 months of age with special reference to the influence of growth and environment under normal conditions of calf management.

Theiler, Green and du Toit (5) reported a value of 6.5 mg. of magnesium per 100 cc. of whole blood for a calf 24 hours after birth. Green and Macaskill (6) found that the concentration of magnesium in the whole blood of a cow and calf was higher than in the plasma and that about two-thirds of the magnesium was in the corpuscles and one-third in the plasma. Normal magnesium values of young calves were found to vary from 2.25 to 2.75 mg. per cent according to Sjollem (7) but the values for calves from 9 to 10 months of age were given as 1.6 to 1.8 mg. per cent. Příbyl (8) found that the serum magnesium varied from 1.21 to 2.24 mg. (average 1.97 mg.) in four suckling calves and that these values were within the same range as their dams.

Allcroft and Godden (9) reported that the serum magnesium values of calves at birth were definitely lower than the normal value for the dam and remained so for the first two weeks. Their mean value obtained from 55 determinations on calves from birth to 28 days of age was 1.79 mg. per 100 cc. (range 1.12-2.25 mg.), whereas the mean value obtained from 32 de-

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terminations during the second month of life was 1.97 mg. (range 1.47–2.50 mg.). Groenewald (10) concluded from a series of determinations that the mean magnesium value was 3.09 mg. per 100 cc. of blood plasma for calves from 1 to 85 days of age (range 2.3–4.2 mg.). He also observed that the magnesium content of calf blood was slightly higher than that of cows' blood and that there was no decline of this element in calves' blood during the first three months of life. Malý (11) reported that the blood magnesium of suckling calves ranged from 1.29 to 2.24 mg. (average 1.72 mg.). We (1) have previously indicated that normal plasma magnesium values varied from 2.25 to 2.75 mg. for calves from birth to 300 days of age. Under the conditions of the experiments reported by Herman (12), the average normal serum magnesium value of calves from 8 to 369 days of age was 3.23 mg., with variations from 2.53 to 4.72 mg.

The present paper is the first report of a series of investigations concerning the normal concentrations, variations, and relationships of certain constituents in the blood of growing and mature dairy animals, in health and disease, and deals specifically with plasma magnesium.

#### METHODS

Several important considerations governed the routine of this experiment. An effort was made to obtain comparable samples of blood from calves which were representative of normal practice in calf management. The blood was always withdrawn in the morning about one hour prior to the regular feeding time so the influence of food was a negligible factor. A uniform procedure was also adopted for the withdrawal of the blood and its disposition after being received in the laboratory. In no instance was the blood allowed to stand at room temperature for any length of time. The routine analytical procedure for the determination of plasma magnesium has been previously reported (1). In nearly all cases it was possible to check the results by a duplicate determination and such checks were invariably within reasonable agreement ( $\pm 5$  per cent or less of the magnesium determined). The significance of the results is further enhanced by the fact that one person (C. C. L.) was responsible for all of the determinations.

Samples of blood from 107 calves (86 Holsteins, 14 Jerseys, 4 Guernseys, and 3 Brown-Swiss, of which 47 were males and 60 were females) were obtained every week or every two weeks. In a few cases less than 10 samples of blood were obtained but in other cases it was possible to obtain the samples during the entire period. The average number of samples from each calf was 21.

*Rations*—The calves used in this investigation received whole milk twice a day from birth to 60–90 days of age in amounts according to the needs of each calf, after which time they were changed to a ration of skim

milk. The skim milk was discontinued at 5-6 months of age. A mixture of corn and oats, equal parts by weight, was fed as soon as the calves would eat it and alfalfa hay (U. S. No. 2) was also fed ad lib. from the same time. Corn silage was fed after the calves were 4 months of age. Water was offered twice a day until they were placed in stanchions at about 1 year of age after which they were watered by individual water bowls. The heifers were turned outside during the day for exercise except during inclement weather. They were also pastured on June grass or alfalfa pasture during the pasturing season after they were 10 months of age.

The protein content of the alfalfa hay averaged 15 per cent whereas the phosphorus content was less than 0.2 per cent.

### RESULTS

The statistical evaluation of the results are recorded in Table 1, supplemented by a histogram (Fig. 1) and a graph (Fig. 2). The data

TABLE 1  
*Effect of age on plasma magnesium*

Age	Deter- mina- tions	Mean	Min.	Max.	S.D.	P.E.	C. of V.
mo.	no.	milligrams per 100 cc. of plasma					per cent
0.5	163	2.389 ± 0.015	1.78	3.14	0.287	0.194	12.01
1.5	259	2.345 ± 0.014	1.64	3.33	0.334	0.225	14.24
2.5	260	2.345 ± 0.015	1.62	3.33	0.360	0.243	15.35
3.5	244	2.322 ± 0.014	1.66	3.27	0.323	0.218	13.90
4.5	230	2.407 ± 0.015	1.67	3.36	0.341	0.230	14.16
5.5	173	2.415 ± 0.020	1.78	3.67	0.385	0.260	15.94
6.5	115	2.429 ± 0.025	1.74	3.65	0.390	0.263	16.06
7.5	112	2.441 ± 0.023	1.66	3.43	0.365	0.246	14.95
8.5	93	2.471 ± 0.028	1.84	3.71	0.400	0.270	16.18
9.5	96	2.428 ± 0.030	1.84	3.75	0.431	0.291	17.75
10.5	98	2.535 ± 0.031	1.71	3.60	0.457	0.308	18.02
11.5	91	2.591 ± 0.032	1.87	3.65	0.449	0.303	17.32
12.5	72	2.589 ± 0.034	1.99	3.63	0.430	0.290	16.60
13.5	67	2.445 ± 0.038	1.83	3.67	0.461	0.311	18.85
14.5	59	2.470 ± 0.037	1.84	3.77	0.424	0.286	17.16
15.5	52	2.403 ± 0.035	1.84	3.83	0.369	0.249	15.35
16.5	51	2.431 ± 0.033	1.97	3.38	0.353	0.238	14.51
17.5	51	2.531 ± 0.036	1.86	3.72	0.384	0.259	15.16
Combined	2286	2.414 ± 0.005	1.62	3.83	0.378	0.255	15.65

presented in the table were derived from 2286 analyses of blood plasma from calves from birth to 18 months of age, and show the number of determinations made for each of the 18 months, the mean magnesium value and its probable error for each month expressed in terms of milligrams per 100 cc. of blood plasma, the minimum and maximum value for each month, the standard deviation, probable error, and the coefficient of variation for each of the periods under consideration.

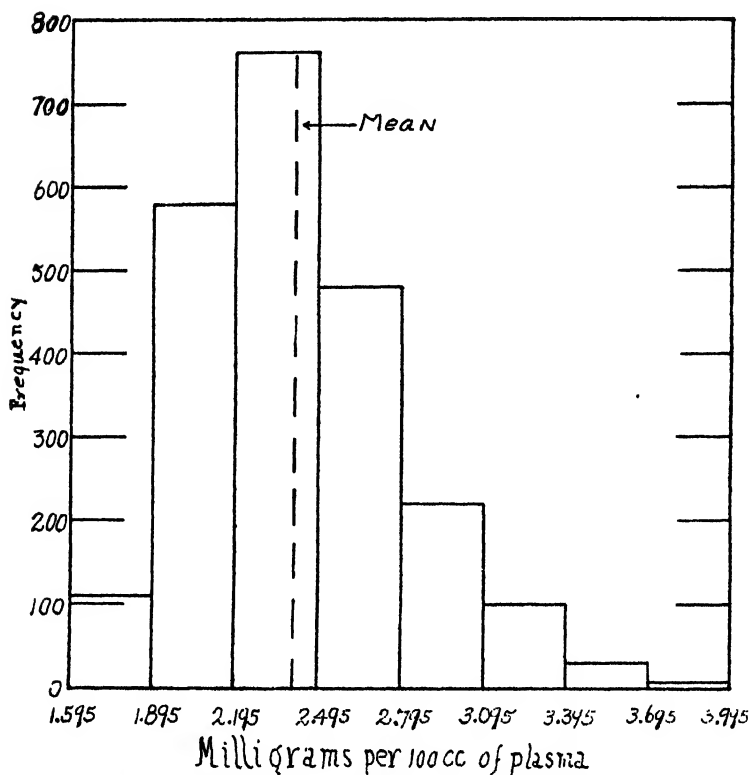


FIG. 1. Histogram showing grouped frequency distribution in variation of 2286 plasma magnesium values from 107 normal dairy calves.

In a frequency distribution table (unpublished basic data) from which the data in Table 1 were derived, the data were classified into 23 classes with an interval of 0.10 mg. between each class. By dividing the data into this number of classes a fairly uniform distribution was obtained. The

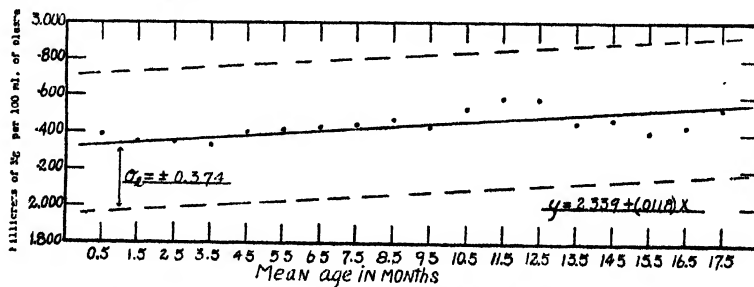


FIG. 2. Dots represent the observed mean plasma magnesium values of normal calves from birth to 18 months of age and the straight line obtained from the experimental data by the method of least squares.

histogram (Fig. 1) was constructed by grouping the data in the frequency distribution table into class intervals of 0.30 mg.

#### DISCUSSION

From an analysis of the results obtained by the repeated determinations of magnesium in the blood plasma of growing calves, it is evident that both the absolute and relative amounts of this element were subject to considerable variation. It will be seen at once that the mean values obtained are at some variance with those recorded in the literature. Our mean values are definitely higher than some that have been reported (7, 8, 9, 11) and definitely lower than others (5, 10, 12). The significance that may be attached to any series of determinations of magnesium in the blood depends largely upon the conditions under which the determinations were made. There are many factors which may affect the values obtained, including the method of analysis and the particular procedure employed in carrying out a given method. When all other conditions are uniform irregularities in handling the blood after it is drawn will give rise to surprisingly large differences in the values obtained. Permitting the blood to stand at room temperature or to be stored in a refrigerator for some length of time tends to increase the magnesium in the blood plasma. It is desirable to emphasize the fact that the results recorded above are to be viewed as results obtained under certain prescribed conditions which differ in many respects from those governing the determinations made by other investigators.

The values obtained for magnesium may be regarded as showing fairly close agreement for the ages under consideration. It is important to state, however, that all of the 47 male calves had been removed from the investigation by the time they were six months of age. The values recorded in Table 1 for the first six months represent the mean values obtained for both male and female calves. The data for each sex were statistically analyzed and no significant differences were noted for the period under consideration. Starting with the seventh month the mean values are for female calves only.

The extreme difference between the lowest and highest mean value (2.322 mg. for the 4th month and 2.591 mg. for the 12th month) is only 0.268 mg. or 11.1 per cent of the mean for the entire 2286 values. The limits of the probable variation from the trend line in Fig. 2 as determined by the standard error of prediction of the combined results are  $\pm 0.374$  mg. per 100 cc. of plasma, whereas the extreme limits of observation indicate an actual difference in the magnesium content of the plasma of normal calves of over 100 per cent.

Another important feature of these results is the array of the observed means about the predicting line. The prediction curve and the dots representing means (Fig. 2) show the goodness of fit of the means of the

observed values to the prediction curve derived by the method of least squares from the correlation table (unpublished). In a normal distribution 68.26 per cent of the experimental values will fall between  $-1 \sigma_p^*$  and  $+1 \sigma_p$ , whereas under our experimental conditions, 72.5 per cent of the observed values actually occurred between  $\pm 1 \sigma_p$ . On the basis of probability, the chances are 2.64 to 1 that any experimental value will be found within one standard error of prediction above or below the trend line. It is also interesting to note that Fig. 2 shows two types of variation. One of these is progressive in character and represents a gradual increase in the magnesium values from birth to 18 months of age. This gradual change in concentration is also accompanied by clearly defined series of rhythmic variations which extend over periods of several months. The first variation occurred during the first 4 months of life during which time there was a slight downward trend. This trend was reversed during the 5th month and the maximum value was established during the 12th month. During the next 6 months the direction was mixed but the mean values for the 17th and 18th months were definitely lower than the 12th and 13th months. The coefficients of variation were highest from the 10th to the 15th month, indicating, probably, a period of physiological disturbances. The figure indicates, however, that neither of these conditions represent a continuous movement in either direction and that high or low values were not maintained indefinitely.

The histogram (Fig. 1) shows fairly close agreement in the frequency with which values of a given magnitude occurred and the amount of asymmetry obtained from the distribution of all values. The coefficients of variation are comparatively large (12.01 to 18.85 per cent) but values between 1.895 and 2.795 mg. of magnesium per 100 cc. of plasma occurred with great frequency (79.7 per cent of all observations), while values below 1.895 and above 2.795 mg. were by no means rare. The extreme limits of observation indicate a potential difference in the magnesium content of the plasma of normal calves of over 100 per cent, whereas the limits of variation between 1.895 and 2.795 mg. indicate a difference of only 37.3 per cent of the mean for all months. This difference is large in absolute value and large in terms of percentage of the mean for all months. It is safe to assume, however, that the mean value obtained for each month indicates an actual difference in the concentration of magnesium in the blood from month to month.

Progressive changes in the chemical composition of the blood can, under certain conditions, be accounted for in various ways, but no satisfactory explanation can yet be offered for the factors which determine the level of magnesium or the factors which cause the periodic variations in the level of plasma magnesium in the growing bovine. The magnesium content of

\*  $\sigma_p$  = Standard error of prediction.

the blood depends primarily upon the intake and utilization of magnesium, its storage and release from the tissues and skeleton and also upon the rate of growth.

#### SUMMARY AND CONCLUSIONS

Determinations of magnesium were made on the blood plasma of 107 normal dairy calves at intervals of 1 to 2 weeks over a period of 3 years. Values were calculated for the mean concentration of magnesium in the blood plasma for the first 18 months of life. The mean magnesium values showed fairly close agreement from month to month and a definite tendency to increase up to 12-13 months of age. The change in level was accompanied by a series of rhythmic variations which extended over several months. The mean value for all of the observed values was 2.414 mg. per 100 cc. of blood plasma (range 1.62-3.83 mg.) and 79.7 per cent of the values were between 1.895 and 2.795 mg. (Fig. 1), whereas 72.5 per cent of the values actually occurred within the limits established by the band of normality (Fig. 2).

The values for magnesium, as recorded above, may be regarded as a cross-section of results obtained from a fair sample of normal dairy calves and may be used as a standard of comparison in estimating the probable significance of other determinations. From the evidence obtained in these experiments, one must conclude that the mean values obtained for the concentration of magnesium in the blood of young dairy animals from month to month differ significantly from values obtained by other workers.

It has been shown that the concentration of magnesium can not be regarded as constant. The range of the so-called normal variation is in all probability sufficiently wide to include many variations that occur under pathological as well as physiological conditions since the maximum and minimum values for each month are definitely outside of the limits of the band of normality established for the combined values of the prediction curve. The results of this investigation also make it evident that fluctuations in the plasma magnesium content of the blood of growing dairy calves are to be expected as normal occurrences.

The authors wish to express their appreciation to Dr. W. D. Baten for his assistance with the statistical treatment of the data.

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# FASTING ENERGY METABOLISM DURING LACTATION\*

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What is the energy requirement for physiologic maintenance<sup>1</sup> in the lactating dairy cow? Important as it is in the evaluation of energetic economy or efficiency of lactation, this question has not been answered satisfactorily in the pertinent literature. The common practice has been to substitute the so called basal metabolism<sup>1</sup> of a dry cow or a steer for the physiologic maintenance requirement of a lactating animal. Yet, it is well known that production of normal milk volume necessitates the feeding of twice to three times the energy required to maintain the dry animal, and that this extra energy intake is not wholly represented in milk energy yielded. We should like to know what part, if any, of this rather huge energy intake is actually necessary to change unit metabolizing tissue from the non-productive to the productive state, and what part is lost as heat increment of feeding. Graham Lusk (1) concluded that there was no difference in the basal energy production<sup>1</sup> in lactating and non-lactating women. But, it seems unbelievable that such can be true also for the dairy cow, an animal in which the persistency and quantity of milk production have been greatly enhanced through generations of selective breeding.

To determine the physiologic maintenance level an animal must be fasted until the specific dynamic effect of the previously ingested food reaches a minimum value. This requires about two days in the ruminant, and causes a decrease in milk yield. The question may therefore be raised as to whether a cow continues to lactate, in the true sense of the word, when subjected to such treatment. Some investigators, in fact, believe that a fasting animal is not a normally lactating animal because of the decline in milk yield. The dairyman on the other hand commonly considers lactation to continue as long as any milk is produced. However, lactation itself is believed to be a physiologic function, whereas milk yield is simply a result of lactation. If, as recent work suggests, lactation is regarded as an endocrine-stimulation of an energy-converting system between milk precur-

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<sup>1</sup> Physiologic maintenance is a term recommended by the Committee on Animal Nutrition, National Research Council, to refer to the energy necessary for maintenance of energy equilibrium under ideal conditions of environment. While physiologic maintenance and basal metabolism are synonymous in that both represent a level of energy production during fasting, the former is preferable for ruminants, since it is doubtful if these animals ever reach a post-absorptive state comparable with humans.

sors and milk constituents then we may reasonably believe that it may persist quite normally during fasting. The latter point of view appears justified for the following reasons: 1, The reduction or even cessation of milk flow by fasting or failure to remove formed milk have no apparent effect, within certain limits, upon the ability of the animal to again yield a normal quantity of milk after removal of the inhibition (2, 3); 2, A falling milk yield is compensated in part at least by changes in the percentages of milk constituents. This is true particularly for milk fat, its position as primary, energy-yielding, milk constituent being increasingly emphasized during fasting.

This report presents preliminary data obtained from fasting energy metabolism studies with a lactating and a dry dairy cow, which were subjected to the same conditions of environment and treatment. The main objectives of these studies were, 1, to ascertain how the fasting heat production curves of the lactating and dry cow were related; and, 2, to find if a physiologic maintenance level could be measured during lactation. As a working basis, lactation was assumed to be an endocrine-stimulated energy-converting system, not necessarily measured by quantity of milk produced. It was further assumed that such an endocrine-stimulated activity may reasonably be placed in the category with other activities such as the circulatory, respiratory, heat regulating, and excretory mechanisms, which are usually considered quite resistant to such physiological stress as fasting.

#### EXPERIMENTAL

The fasting heat production of an eight year old Guernsey cow in the fourth month of lactation and an eight year old Jersey cow which had been dry for over a year was measured by an open circuit-mask respiration method which has been described previously (4). Energy metabolism was computed from the respiratory quotient<sup>2</sup> and volume of directly expired air obtained from the animals in a lying position, in respiration periods of 20-30 minutes duration. The respiration periods were started as soon as possible after consumption of the last feed, and were repeated about every two hours during the first 12 hours of fast and about every 3 to 5 hours thereafter. Corresponding periods for each animal were carried out at approximately the same time of day or night and interval after feeding.<sup>3</sup> Prior to fasting each animal was given feed of equal quality and quantity. Between respiration periods the animals were kept in adja-

<sup>2</sup> Total respiratory quotient, uncorrected for urinary nitrogen, but corrected for fermentation methane and carbon dioxide, determined by gas analysis with Haldane apparatus.

<sup>3</sup> Immediately after ending a period on one cow the other animal was started. About 5 minutes intervened between the periods of each animal to insure complete washing out of apparatus for gas sampling.

cent metabolism stalls (5), and were at all times subjected to the same manner of handling and environmental temperature (thermal neutrality). The lactating cow was milked regularly at 6 o'clock a.m. and p.m.

The resulting data are presented in Fig. 1 and Tables 1, 2, and 3.

TABLE 1

*Average daily live weight, food and water consumption and excreta production during preliminary feeding periods*

Animal	Live Weight	Hay <sup>a</sup>	Silage <sup>b</sup>	Grain <sup>c</sup>	Water	Milk	Feces	Urine
	Kgs.	Kgs.	Kgs.	Kgs.	Kgs.	Kgs.	Kgs.	Kgs.
#428 Lactating	373.2	4.709	11.693 8.711*	3.178 1.589*	28.820	6.171	19.866	7.556
#831 Dry	504.1	2.954	10.536 6.910*	3.178 1.589*	26.199		18.289	8.657

<sup>a</sup> 5.0 Kgs. good quality alfalfa hay offered at p. m. feeding.

<sup>b</sup> 13.0 Kgs. silage offered at a. m. feeding.

<sup>c</sup> 1.589 Kgs. grain mixture offered at a. m. and p. m. feedings.

\* Last feed before fast. Average for Experiments 2 and 3.

TABLE 2

*Average milk and fat production during fasting  
(Average for 3 trials)*

Period	1	2	3	4	5	6
Hours fasting	12	24	36	48	60	72
Milk (Gms.)	2765	3111	2026	2006	1262	1612
Fat (Gms.)	134.9	153.1	135.0	136.8	98.4	137.0

## DISCUSSION

While these data leave much to be desired, they point to some interesting aspects of energy metabolism in the dairy cow. Figure 1 shows that changes in fasting heat production are in general similar for both cows. In both animals the energy metabolism declines to about 47 per cent of the initial value within 48 hours after the last food is consumed. The absolute heat production (top chart) is about the same for a 750-pound lactating cow and a 1100-pound dry cow, each receiving the same kind of feed to the limit of appetite. Compared on a physiological weight basis (7), the heat production (middle chart) by the lactating cow is of the order of 20 per cent higher than that of the dry cow. This difference remains constant up to 60 hours fasting. Corrected further for gross energy intake (bottom chart), the energy production curve for the lactating cow is 10 per cent higher than that for the dry cow. Any attempt to interpret curve II (bottom chart) would be idle speculation with the data at hand. This curve may indicate that in the lactating cow, 1, an increasing rate of energy

TABLE 3

*Average daily body weight loss, nitrogen excretion (milk and urine), and fecal output during fasting*

Period of fasting	Weight loss Kgs. per 72 hrs.	Nitrogen excretion Gms. per 24 hrs. <sup>a</sup>	Fecal output		
			Kgs. dry matter per 24 hrs. <sup>b</sup>		
			Ballast <sup>c</sup>	Feces	Per cent loss
		# 428 Lactating Cow			
First 12 hrs. ...		91.990	10.979	4.131	37.6
12-36 hrs. ....		87.439	6.848	2.603	38.0
36-60 hrs. ....		41.360	4.245	1.271	29.9
60-72 hrs. ....		71.972	2.974	0.502	16.9
Average .....	25.117	66.924 (12-72 hrs.)			
		# 831 Dry Cow			
First 12 hrs. ....		129.122	8.507	3.605	42.4
12-36 hrs. ....		83.142	4.902	1.437	29.3
36-60 hrs. ....		66.509	3.465	0.342	9.9
60-72 hrs. ....		51.633	3.123	0.137	4.4
Average . . . . .	26.346	67.095 (12-72 hrs.)			

<sup>a</sup> Uncorrected for specific gravity—assuming 1 gm. per cc. of urine or milk.

<sup>b</sup> Dry matter computed as follows: hay, 90%; grain, 90%; silage, 30%. Fecal dry matter assumed to be same for lactating and dry cow; about 20.1%. Also assuming that dry matter percentage remains fairly constant during fasting. See Benedict & Ritzman (6).

<sup>c</sup> Initial ballast assumed to be proportional to: *Daily dry matter intake - Daily dry matter of feces + Dry matter intake of last feeding*. Ballast for succeeding periods computed by subtracting fecal output from ballast in preceding period.

utilization occurs after 36 hours fast; or, 2, that heat increment per unit energy absorbed increases, possibly along with a declining demand for maintenance energy; or, 3, that body tissue becomes increasingly more important as a source for milk energy. In this connection it might be stated that loss in weight during fasting was apparently no greater for the lactating cow than for the dry cow. This is shown in Table 3 and further substantiated by the nitrogen excretion values in the same table.

A striking difference in the energy production of these cows is shown by the break in the curve for the lactating animal occurring after 60 hours of fast. The heat production level reached by both cows at 36-48 hours is maintained at least until 72 hours by the dry cow, but in the lactating cow it further declines about 20 per cent between 60 and 72 hours. This drop in total metabolism of the lactating cow at first thought seems fortuitous, but when considered in the light of other data presented herein it takes on significance. Table 3 shows that at 72 hours after food the lactating cow excreted feces at a rate about 400 per cent greater than the dry cow. Since these animals continued to excrete considerable quantities of methane,<sup>4</sup> even

<sup>4</sup> Methane excretion variable. Average value about 1.5 liters per hour between 36 and 72 hours fasting.

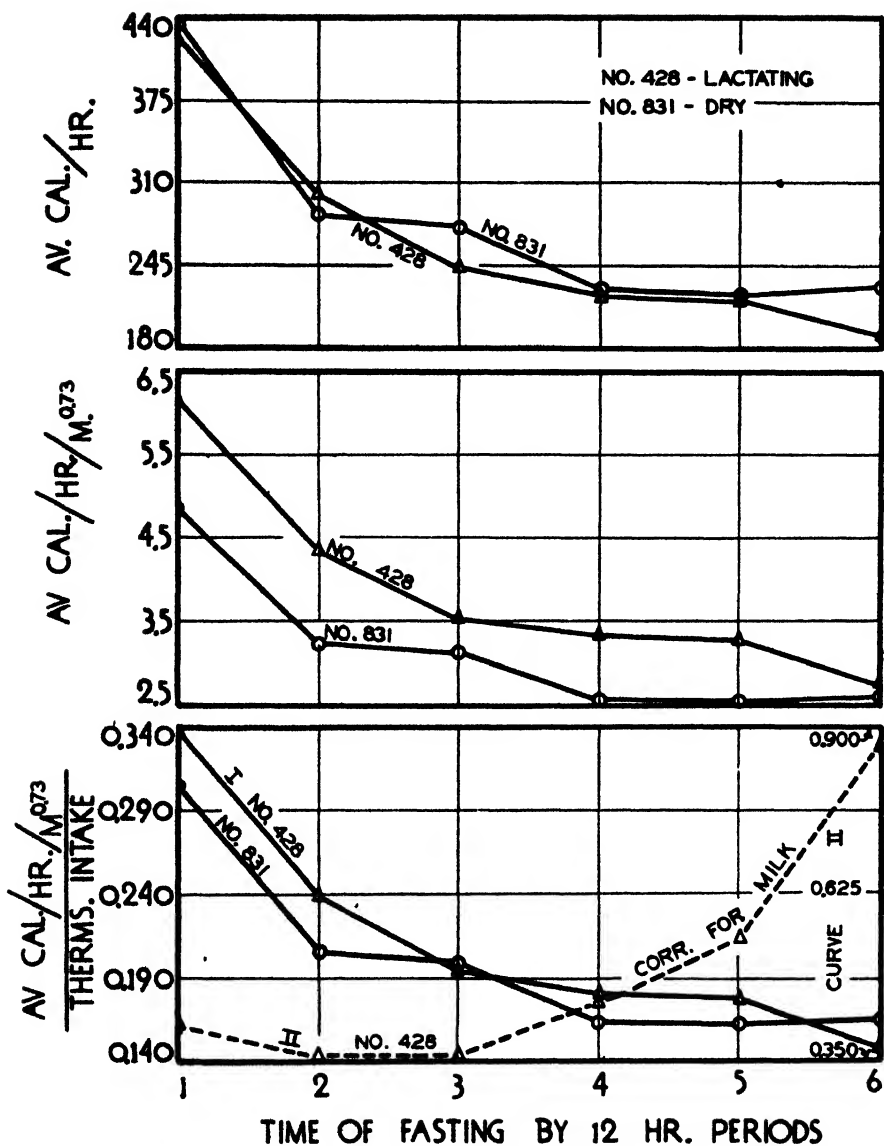


FIG. 1. Fasting heat production curves for lactating Guernsey cow No. 428 and dry Jersey cow No. 831. Top chart—total heat production (avg. for 2 fasting experiments). Middle chart—heat production per unit physiologic weight (0.73 power of live weight in kilograms). Bottom chart—values in middle chart further corrected for differences in energy intake (Therms). Curve II for cow No. 428 obtained by dividing values given in middle chart by gross energy intake minus milk energy (Therms).

at 72 hours, it appears that fermentative digestion and absorption were going on. Thus a certain part of the total heat production, even after a level had been attained, might have been due to heat increment of nutri-

ment absorbed from alimentary residue. The rapid rate of evacuation of the digestive tract by the lactating animal would then result in a decline in absorption and a drop in total heat production. In other words the lactating cow might have been approaching the so called hypothetical minimum energy production more quickly than the dry cow. Inasmuch as the hypothetical minimum metabolism of the ruminant has never been reported, we do not know how to apportion the total heat production during fasting between heat increment and the so called tissue metabolism.

It does not seem impossible that, under the strain of fasting, extra-mammary systems in the lactating animal might compensate by subsisting on a lower maintenance plane, so that mammary activity might be prolonged. Despite the common opinion that lactation stimulus elevates the level of energy metabolism of body tissue in general, no evidence has yet been advanced to show whether the increased total metabolism in lactation is due to elevated tissue metabolism or to increased heat increment of feeding. Apparently, increased food consumption is a factor in the higher metabolism of lactating cows stimulated by thyroxine (8, 9). It is well known that thyroxine stimulation in humans and other animals also results in greater food intake and tissue breakdown. Moreover, recent observations indicate that secretions of the pituitary (10, 11) and adrenal glands (12) act upon the alimentary system and are important factors in the absorption of nutriment.<sup>5</sup> Is it not justifiable then to assume that the endocrine stimulus of lactation acts in a large degree upon the alimentary system, making for greater nutriment absorption and hence a greater heat increment from nutriment? Of course it can be argued that greater food intake in lactation is caused by a greater demand of body tissue for energy. In this connection, however, one must remember that food intake is regulated essentially by appetite or hunger manifested by characteristic sensations and activity of the alimentary system, not by body tissue in general.

Another possible cause for the drop in total metabolism of the lactating cow might be the breakdown and disappearance of some metabolism stimulating mechanism associated with lactation. It is possible that at 60 hours the heat production of the lactating animal begins to approach that of the dry animal, finally reaching such a level upon complete cessation of milk secretion. The data in Table 2, however, do not seem to substantiate this reasoning, at least within the limit of 72 hours of fast. It appears that the fat synthesizing mechanism endured with remarkable constancy during the entire experimental period. Furthermore, when corrected for energy intake in the feed the total heat production of the lactating animal drops below that of the dry animal at 72 hours.

<sup>5</sup> While thyroxine and the pituitary and adrenal hormones are referred to in this connection merely as an example, they are also thought to be important factors in lactation stimulus.

## SUMMARY

Preliminary data obtained by an open-circuit-mask respiration method indicate that the total heat production of a lactating cow during fasting is about 10 per cent higher than that of a dry cow. Up to 60 hours of fast the heat production curves of both animals are essentially parallel, reaching a level at about 36 to 48 hours after feed. After 60 hours the heat production of the lactating animal further declined about 20 per cent. Certain data indicate that the higher level of total energy metabolism in the lactating cow is in a large measure due to heat increment of nutriment.

During 72 hours of fast, the lactating cow continued to produce a relatively constant amount of milk fat, although her milk yield declined about 50 per cent. It is believed, therefore, that within the limits of these experiments, lactation as a mechanism was unchanged by fasting.

Further investigation of digestion, absorption, and incident heat increment of nutriment appears to be necessary in order to evaluate true physiologic maintenance in the fasting ruminant. The probability of considerable absorption occurring 72 hours after food, suggests that the fasting level of total heat production is not an accurate measure of physiologic maintenance in the ruminant. Furthermore, differences in the speed of movement of alimentary residue during fasting, indicate that the fasting levels of total heat production in the lactating and dry cow are not comparable without further correction.

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# EFFECTIVENESS OF ULTRAVIOLET LIGHT APPLIED TO THE HEAD OR BACK REGIONS OF CALVES

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While considering an installation of ultraviolet lamps in a dairy barn the question arose as to the position of the lamps with respect to the cow's body. It was known that solar irradiation of the cow increases the vitamin D content of milk (1) as does irradiation with artificial sources of ultraviolet light (2, 3, 4) although the evidence on this point is conflicting (5, 6). Calves undoubtedly can use ultraviolet from solar or artificial radiation to alter their blood chemistry and mineral metabolism to such a degree as to prevent or cure rickets (7, 8, 9). From the work just cited, however, there is no means of determining what regions, if any, of the cow's body are particularly penetrable to ultraviolet light. In other species marked differences in the effectiveness of ultraviolet have been demonstrated by directing the radiation at different body sectors (10, 11, 12).

Of particular interest in this respect is the work of Knudsen (13) who showed that in rats radiation of the head region was much more effective than radiation of the back. He also found that the hair on the back probably was preventing absorption of the ultraviolet because when shaved areas were exposed marked antirachitic effects were obtained.

As preliminary, the work of Knudsen was partially repeated. Rachitic rats were irradiated for various periods with a Cooper-Hewitt lamp at a distance of 30 inches. In addition to substantiating the work of Knudsen the data in Table 1 suggest the significant point that if the radiation is continued long enough considerable ultraviolet penetrates the unshaved back.

TABLE 1  
*Response of rachitic rats to ultraviolet light*

Part of body exposed	Length of exposure	No. of rats	Av. line test
	<i>min.</i>		
Head	120	2	3.00
Back (unshaved)	120	2	0.75
Controls		2	0.00
Head	60	4	0.88
Back (unshaved)	60	4	0.38
Controls		4	0.00
Head	30	2	0.00
Back (unshaved)	30	2	0.00
Back (shaved)	30	5	1.20
Controls		3	0.00

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These findings with rats prompted a study with calves which, it was hoped, might throw some light on the question of placement of ultraviolet lamps in a dairy barn installation.

#### EXPERIMENTAL

Three groups of three calves each were used in this experiment. All the calves received whole milk in amounts sufficient to meet their needs during the first few weeks. They were then gradually shifted to liquid skimmilk which was fed at a maximum of 16 pounds daily. The calves were fed a grain mixture composed of equal parts ground yellow corn and ground oats. At the end of 120 days skimmilk feeding was discontinued. Water and salt were given free choice to all calves. The calves were penned in groups of three, each pen being provided with stanchions so that the animals could be treated as individuals. Raised slatted floors eliminated the use of bedding.

The calves in Group I were irradiated directly on the face and head by ultraviolet lamps placed in front of the head position of each calf as it stood in a stanchion. The calves in Group II were irradiated on the back by lamps placed above the center of the back region of each calf as it stood in a stanchion. In order to restrict the treatment to the desired region each group of calves was draped with a black cloth which prevented the light from striking anywhere but on the head region of the calves in Group I and screened the head region from the light in Group II. Group III served as controls and received no ultraviolet light. The lamps used were of special design to meet the needs of the set-up.<sup>1</sup>

Measurements were made monthly with an ultraviolet meter of the light intensity on the heads and backs of the respective groups. Readings were made at several points of the head and back areas. Any required equalization of ultraviolet intensity was accomplished by adjusting the height of the lamps in the back-irradiated group. The average intensity of ultraviolet light reaching the calves' heads was 82, and that reaching the calves' backs 84, micro-watts per square centimeter. The calves in Groups I and II were exposed to the lights for 45 minutes daily, including a 10 minute warming up period. It is estimated that the daily exposure to ultraviolet light was approximately equal to 2 hours of midsummer sunshine.

At the beginning of the experiment, and at approximately monthly intervals thereafter, blood samples were taken from each calf and the amount of calcium and phosphorus in the serum was determined. The Clark-Collip method for calcium (14) and the Bell-Doisy-Briggs procedure for phosphorus (15) were followed. At the same time blood from the calves of each group was pooled and dried before fans in a hot air chamber. These samples of dried pooled blood were later used for vitamin D comparisons, using the line test procedure.

<sup>1</sup> The lamps were furnished and the installation and ultraviolet measurements made by the General Electric Co., Nela Park, Cleveland, Ohio.

**TABLE 2**  
*Calcium and phosphorus in blood serum of calves*  
(Mg. per 100 cc.)

Date	Head group						Back group						Control group					
	1G		2H		3H		4H		5J		570H		6J		569H		581H	
	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P
11/16/36	10.99	10.45	11.44	9.40	10.69	8.51	10.59	7.96	10.99	10.45	10.54	8.83	11.34	9.87	11.14	9.17		
12/14/36	9.54	8.20	10.59	9.40	10.74	8.08	10.89	8.08	11.24	10.25	9.34	8.60	10.04	6.99	6.48	9.93		
12/23/26																	11.75	10.80
1/11/37	Dead		10.34	10.00	10.19	8.93	10.89	9.25	10.84	9.57	6.83	8.53	5.82	9.94	5.47	7.88	10.34	9.13
1/21/37											6.98	6.77			4.62	8.65		
2/ 4/37															5.22	7.20		
2/ 8/37			10.79	8.98	10.57	8.26	9.90	8.05	11.14	8.73	Dead		5.57	8.37			8.73	9.33
3/ 9/37			11.10	7.73	10.56	8.18	10.47	7.40	10.13	8.88			5.67	7.47	Dead		5.72	8.33
4/ 7/37			10.42	8.37	10.27	6.70	9.34	7.50	8.85	7.73			6.31	5.56			6.31	7.57
5/14/37			Dead		Dead	Dead	10.22	6.95	Dead				Dead				5.86	5.42
							Killed										Killed	

After each calf died or was killed, the front and rear cannon bones and both eighth ribs were removed and freed of adhering flesh. The breaking strength of the left front and rear cannon bones was determined on an Olsen breaking machine, and the ash content of the distal end (one-third of the length) obtained after extraction with alcohol and ether. The same procedure was followed in determining the ash content of one-tenth of the rib bones, measured from the costochondral end.

The blood calcium and phosphorus data are presented in Table 2. Calf 1 G (head group) refused to eat and died, probably from starvation, early in the trial. Of outstanding significance is the difference between the values for Groups I and II and those of Group III. The control calves developed a blood picture indicative of rickets of the low calcium type. The blood calcium and phosphorus values of the irradiated calves are for the most part within the range of normality except for Calf 570 H, which died at the age of 2½ months from an injury obtained during a violent convulsion. At the time of death the calcium level of this calf's blood was rather low. The other two calves in this group showed normal blood pictures at the time of the death of 570 H. This fact, plus the rather variable results for calcium on calf 4 H, indicating possibly that it was on the borderline, and the somewhat lower calcium value on 5 J shortly before it died, indicates that the back-treated calves did not respond quite as well as did the head-treated calves.

The data in Table 3 substantiate this to some extent. During the first part of the experiment the vitamin D potency of the pooled blood from the

TABLE 3  
*Vitamin D potency of dried blood (line test procedure)*

Date	Amount fed	Line test values		
		Head	Back	Control
	<i>mg.</i>			
11/16/36 .. . .	500	0.0	0.0	0.0
	1000	0.0	0.0	0.0
	2000	0.0	0.0	0.0
	3000	0.0	0.0	0.0
1/11/37 .... . . .	2000	0.25	0.0	0.0
	3000	0.50	0.15	0.0
2/ 8/37 .. . .	2000	0.70	0.17	...
	3000	.....	....	0.0
	4000	.....	.....	0.0
3/ 9/37 ..... . .	2000	0.53	0.64	.
	3000	....	.....	0.0
	4000	.....	.....	0.0
4/ 7/37 ..... . . . .	2000	0.13	0.25	.....
	3000	0.20	0.25	0.0
	4000	.....	.....	0.0

head-irradiated calves was definitely greater than that of the back-irradiated calves, but towards the end of the experiment there was no measurable difference. This might indicate that at first radiation of the head region was more effective, but as the treatment continued the calves in the back group caught up. It must also be considered that Calf 570 H, whose blood had been running low in calcium, did not contribute blood to the last two pooled samples.

TABLE 4  
*Ash content and breaking strength of bones*

Group	Calf	% ash in bones		Days on exp.	Breaking strength of cannon bones		
		Leg	Rib		Front	Rear	
		First analysis—All calves considered					
Head	1	59.52	64.53	58	800	903	
	2	58.75	53.02	163	1195	1700	
	3	58.53	50.95	148	1135	1458	
	Av.	58.93	56.17		1043	1354	
Back	4	58.89	52.10	186	1035	1472	
	5	55.63	49.56	154	864	1100	
	570	52.12	41.12	70	846	1405	
	Av.	55.55	47.59		915	1326	
Control	6	54.30	34.98	146	812	1142	
	569	54.27	46.84	82	1182	1372	
	581	51.71	35.67	148	736	1136	
	Av.	53.43	39.16		910	1217	
		Second analysis					
		Calves on experiment 5 months or longer					
Head	2	58.75	53.02	163	1195	1700	
	3	58.53	50.95	148	1135	1458	
	Av.	58.64	51.99		1165	1579	
Back	4	58.89	52.10	186	1035	1472	
	5	55.63	49.56	154	864	1100	
	Av.	57.26	50.83		950	1236	
Control	6	54.30	34.98	146	812	1142	
	581	51.71	35.67	148	736	1136	
	Av.	53.01	35.33		774	1139	

The over-all effect of the various treatments is shown by the data in Table 4. These data may be treated in two ways: 1) when all calves were included; 2) when only those calves on experiment 5 months or longer are included. When the data on all calves are included an appreciable difference is shown between the bone ash content of the head-irradiated group and the back-irradiated group, although no difference in breaking strength is apparent. Again the inclusion of data from Calf 570 H is responsible for the apparent poorer performance of the back-irradiated group. When the data from the calves on trial for 5 months or longer are considered the difference in bone ash between the head group and back group is not signifi-

cant, but breaking strength values favor the head group. No matter which method of treatment is applied both the head and back groups show much better skeletal development than the calves in the control group. This superiority must be attributed to the light treatment.

#### DISCUSSION

Lower blood calcium and phosphorus, decreased bone ash and breaking strength, lack of vitamin D in the blood, enlarged knees and rib ends, stiffness, and a humped back posture, were symptoms in the control calves indicative of rickets. All the calves exhibited anorexia and failed to grow after skimmilk feeding was discontinued. The feeding of dry yeast did not increase grain consumption.

Most of the calves exhibited tetanic convulsions towards the latter part of the experiment. In a few instances following the spasm the calf would pass into a coma and die, or, if the coma was prolonged, the animal was killed. Magnesium determinations made on the blood just previous to or during a convulsion or coma gave values in most instances under 2.0 and as low as 1.6 mg. per 100 cc. These are below the normal values given for calves by Duncan, Huffman, and Robinson (16). The production of low-magnesium tetany was probably due to insufficient magnesium intake and could not be associated with the light treatment.

So far as practical application of these findings is concerned it would seem that a satisfactory installation of ultraviolet lamps would be one which utilized the greatest possible exposed surface. It would need to be assumed, of course, that cows utilize the ultraviolet in the same manner as do calves.

#### CONCLUSIONS

Ultraviolet light from artificial sources, of sufficient intensity to equal approximately the radiation received from two hours of midsummer sunshine daily, is effective in preventing rickets in young calves fed a rickets-producing ration.

Ultraviolet light applied to the region back of the withers and with the greatest intensity on the center of the back, is almost as effective as when an equivalent amount is applied to the head region of calves.

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# NATURE OF THE SWELLING IN THE UDDER OF A COW AT CALVING TIME

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Dairy cows differ greatly with respect to the amount of swelling in and near the udder at calving time. In some cases the udder undergoes a moderate increase in size but otherwise little visible change. In others it becomes swollen—sometimes to the point of extreme distortion. Not infrequently a “plastic” condition develops that may be limited to the lower portion or may involve a large part of the surface of the udder. The term “plastic” is used to denote a condition wherein localized external pressure, as with the fingertips, leaves a persistent indentation. This condition is often referred to as “pitting.” A pronounced case is illustrated in Fig. 1. This kind of swelling usually is cool to the touch. Sometimes the swelling



FIG. 1. Udder with pronounced “plastic” swelling at calving time. Note the persistent indentations resulting from pressure with finger tips. This condition is often referred to as “pitting.”

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disappears in a few days. Sometimes it persists in some degree for several months.

There is much to indicate that the kind of swelling described is the result of edema. By definition edema is an infiltration of serum in a part. A medical dictionary<sup>2</sup> lists 25 or more different types of edema that occur in humans. It seems to be caused by a great variety of disturbances to the physiological functions of the individual. Lymphedema is a swelling of soft tissues resulting from an increased quantity of lymph. Stoppage of lymph circulation, which is known as lymph stasis, results chiefly from some obstruction which causes the lymph to seek new channels. It appears to be conducive to fibrosis (development of fibrous tissue) which produces further stasis and still more fibrosis.<sup>3</sup> It might be expected, therefore, that repeated edematous swelling would tend to produce large, fibrous udders in cows with advance in age.

An edematous condition involving the udder and sometimes extending forward to the umbilicus and more rarely to the brisket, has been found by Parshall<sup>4</sup> in certain cases of acute gangrenous mastitis. It was found accompanied by coagulated blood-like material in the cisterns, multiple abscesses, blood clots in the large veins, gas pockets in scattered areas and necrosis. This condition, however, occurred most frequently between the second and sixth months of lactation. One case was found in a dry cow and only one in twelve occurred at the time of parturition. Bacteriological examination of the udders showed a mixed infection of *Staphylococcus aureus* and some anaerobes of which *Clostridium welchii* was the most important. Injections of pure cultures of either one of these organisms produced only mild cases of mastitis, but injections of both together resulted in marked edema on the day following injection, and typical gangrenous mastitis in 3 of 6 cows inoculated.

Correspondence has recently been carried on with authorities on edema and on lactation in women to learn whether or not a condition analogous to that found in cows at calving time occurs in the breasts of women at child-birth. One reply states that such a condition has not been observed in women. Another indicates that occasionally some edematous swelling does occur in the human breast at parturition, which is thought to be a lymphatic condition. Apparently it does not occur with sufficient frequency or severity to be considered a serious disturbance in the human.

As a rule udders showing an abundance of the swelling incidental to calving, are not available for post-mortem anatomical study, as dairy cows are

<sup>2</sup> Gould's Medical Dictionary (Scott) Second Edition. P. Blakiston's Son & Co. Philadelphia, Pa. 1928.

<sup>3</sup> Allen, E. V. and Ghormley, R. K. Lymphedema of the Extremities. *Annals of Internal Medicine*, 9, No. 1, pp. 516-539. 1935. From the Mayo Clinic.

<sup>4</sup> Parshall, C. J. Nature of Experimentally Produced Gangrenous Mastitis in Cows. *Cornell Veterinarian*, Vol. 24, April 1934. pp. 146-155.

seldom slaughtered at such an early stage of lactation. However, an opportunity was provided for slaughtering a cow that was showing at the time, an extremely congested condition of the udder. The cow was No. 848, a registered Holstein-Friesian, bred and raised in the herd of the Bureau of Dairy Industry, Beltsville, Md.

The usual procedure with all heifer calves in the breeding herd of the Bureau of Dairy Industry, at the Beltsville, Md., Station, is to commence periodic examinations of the comparative development of the mammary gland at an early age. At each examination the status of mammary gland development is graded. Nine grades, ranging from 1 to 9, are used for this purpose. A grade of 1 indicates an extremely retarded development, grade 9 is applied to the most advanced development, grade 5 represents the average development, grades 2, 3 and 4 are below average and grades 6, 7 and 8 are above.

As a young calf the mammary development of No. 848 was definitely below average. Grades of 3 were assigned both at 16 days and at 1 month 27 days of age. At 3 months 2 days the glandular development was graded 2 and the condition was noted as follows: "Udder tissue development appears to be very much retarded. Still in the straight tube stage. About the same development usually found at 2 weeks of age." At all subsequent observations up to the age of 1 year the stage of mammary gland development was below average though some progressive improvement was made and at 18 months the mammary development was definitely above the average. At each observation the udder was characterized by uniformity in the development of the individual quarters and at each examination made between the ages of 9 and 18 months inclusive, the glandular tissue was well attached to the abdominal wall.

Four examinations of the udder were made (Sept. 11 to October 26, 1931) previous to her first calving on November 3, 1931. At the first examination some swelling was noticeable, a grade of 3 for quantity being assigned. The quantity increased steadily, a grade of 5 being given on October 6, a grade of 8 on October 21, and a grade of 9 on October 26—8 days before calving. On October 26 this cow also showed a "very large area of abdominal swelling". After calving the swelling diminished rather steadily, only a very small quantity remaining on February 11, 1932, and only a trace on subsequent monthly observations until August 18, after which it appears to have disappeared entirely. The swelling was moderately plastic in character from October 21 to November 18, 1931.

Udder examinations were not made during the second or third lactation periods, but photographs taken near the time of each calving showed a strong tendency for this cow to develop swelling in the udder at each calving. This was particularly the case at the time of the fourth calving when the size of the udder reached extreme proportions. (Fig. 2.)

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The usual procedure with all heifer calves in the breeding herd of the Bureau of Dairy Industry, at the Beltsville, Md., Station, is to commence periodic examinations of the comparative development of the mammary gland at an early age. At each examination the status of mammary gland development is graded. Nine grades, ranging from 1 to 9, are used for this purpose. A grade of 1 indicates an extremely retarded development, grade 9 is applied to the most advanced development, grade 5 represents the average development, grades 2, 3 and 4 are below average and grades 6, 7 and 8 are above.

As a young calf the mammary development of No. 848 was definitely below average. Grades of 3 were assigned both at 16 days and at 1 month 27 days of age. At 3 months 2 days the glandular development was graded 2 and the condition was noted as follows: "Udder tissue development appears to be very much retarded. Still in the straight tube stage. About the same development usually found at 2 weeks of age." At all subsequent observations up to the age of 1 year the stage of mammary gland development was below average though some progressive improvement was made and at 18 months the mammary development was definitely above the average. At each observation the udder was characterized by uniformity in the development of the individual quarters and at each examination made between the ages of 9 and 18 months inclusive, the glandular tissue was well attached to the abdominal wall.

Four examinations of the udder were made (Sept. 11 to October 26, 1931) previous to her first calving on November 3, 1931. At the first examination some swelling was noticeable, a grade of 3 for quantity being assigned. The quantity increased steadily, a grade of 5 being given on October 6, a grade of 8 on October 21, and a grade of 9 on October 26—8 days before calving. On October 26 this cow also showed a "very large area of abdominal swelling". After calving the swelling diminished rather steadily, only a very small quantity remaining on February 11, 1932, and only a trace on subsequent monthly observations until August 18, after which it appears to have disappeared entirely. The swelling was moderately plastic in character from October 21 to November 18, 1931.

Udder examinations were not made during the second or third lactation periods, but photographs taken near the time of each calving showed a strong tendency for this cow to develop swelling in the udder at each calving. This was particularly the case at the time of the fourth calving when the size of the udder reached extreme proportions. (Fig. 2.)

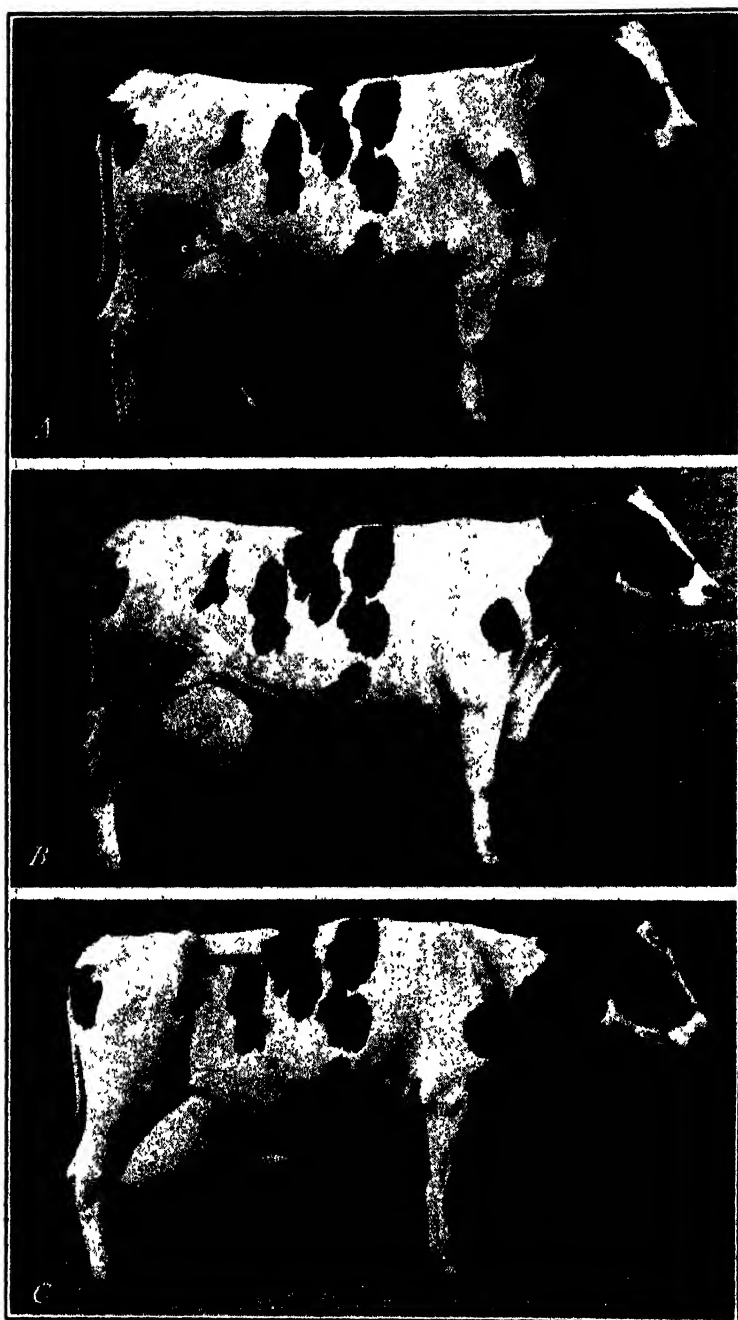


FIG. 2. Cow No. 848 showing edematous swelling on udder and abdomen. A. Five days before calving as a 3-year old. B. Seven days after calving as a 4-year old. C. One day after calving as a 5-year old.

Comments made by the men who milked this cow both by hand and by machine are interesting. She was milked by hand during a large part of her third lactation. The milker states that it was not particularly difficult to squeeze the milk out of the teats but that owing to the short teats and the hard swollen layer at the lower part of the udder, the milking had to be done by stripping between the thumb and finger and 25 to 30 minutes was required for milking. He volunteered the opinion that the swollen condition existing 5 days after calving would have been present to nearly the same extent 5 months after calving, as her udder always stayed hard on the bottom. The machine operator who milked the cow during her first lactation stated that this cow's udder was always hard.

The cow was slaughtered June 11, 1935, after her fourth calving which took place on June 6. Examination of the udder after milking on the morning of slaughter showed it to be of extreme size and to have a very large amount of swelling of a plastic nature. The udder was comparatively poor in shape as Figure 2 shows. It was amputated as quickly as possible after death, and subsequently was filled with formalin and frozen according to the plan regularly followed with cows slaughtered at the Beltsville Station. Although milked out before the cow was killed, the amputated udder weighed 165.65 pounds. This represents 10.9 per cent of the live weight of the cow on June 7—the day the last photograph in Fig. 2 was made.

The procedure followed in post-mortem studies of the udder is to fill the two quarters on one side of the udder simultaneously with formalin under constant pressure. Comparatively few cases (not more than 20 per cent) require more than  $7\frac{1}{2}$  minutes to fill the two quarters of the udder, but owing to the extreme size of this udder the filling was continued for  $12\frac{1}{2}$  minutes. At the end of that time 24,580 cc. of formalin had flowed into the two right quarters of the udder. Assuming that both halves of the udder had the same capacity the entire udder would have held 49,160 cc. which is equivalent in volume to 111.72 pounds of milk—the calculated capacity of this udder. As soon as filling was terminated the left half (unfilled) was separated from the right half by cutting along the left side of the median septum. The nature of the swelling could be clearly discerned when this incision was made. Apparently it was outside and separate from the glandular tissue, which seemed to be unaffected. The layer of swelling was about 2 inches thick. Describing its appearance is somewhat difficult but it seemed to consist chiefly of clear transparent fluid through which ran a net-work of fine silky fibers, glistening in appearance, that resembled spider's web. The fluid was held in some manner by this lacy structure and no appreciable amount escaped at the time the incision was made. The right half of the udder was immediately placed in a freezing room at a temperature of approximately  $10^{\circ}$  F., where it was kept until March 11, 1936. On that date it was cut with a band saw into vertical transverse sections approximately 1 inch thick.

After having been kept in a refrigerated room for 12 months the appearance of the layer of swelling was essentially unchanged, the fluid still being held in some manner by the tissues or by the lacy structure noted on the day of slaughter. Apparently the swelling was edematous in character, and the swollen material will subsequently be referred to as edematous tissue. Photographs of the cut surface of these sections were made according to our usual procedure on June 12, 1936. Although these photographs showed the glandular tissue in splendid detail, the lacy structure within the edematous tissue was less clearly brought out. The structure in the vertical transverse plane directly above the rear teat is shown in Fig. 3.

One of the sections was again photographed on March 30, 1937, more than 21 months after the cow was slaughtered, in an attempt to show the structure of the edematous tissue in greater detail. Even after this extended period the tissue did not appear to be appreciably changed. The appearance of both the glandular and edematous areas in this section is shown in Fig. 4, which represents a vertical plane directly above the front teat. The line of separation between the two areas is quite distinct, although there is some indication of a trace of edema in the strands of fiber running through the glandular tissue along the lower edge of the glandular area. To give an idea of the quantity of swelling present it is noted that the distance from the tip of the teat to the end of the skin covering the udder shown at the upper left, measured  $17 \frac{1}{16}$  inches. Attention is called to the manner in which the hide is pushed away from the glandular tissue, the way in which the right and left halves of the udder have been pushed away from each other, and the extent to which the teat is surrounded and seemingly shortened by the edematous swelling. The apparent shortening of the teat is shown to even better advantage in Fig. 3.

The tenacity with which the fluid was held by the edematous tissue is shown by the lack of change which occurred during the 21 months following slaughter, more than a year of which was after the udder was sectioned. When the udder sections were exposed to warm air, however, there was an evaporation of the suspended fluid and the areas that had appeared to consist primarily of fluid came to resemble a mass of loosely connected fiber.

Small blocks of the fresh tissue were removed on the day of slaughter from the half of the udder not filled with formalin, to be used for histological study. Blocks No. 1 and No. 2 were located  $3\frac{1}{2}$  and  $10\frac{1}{2}$  inches respectively above the front teat; and No. 3, No. 4 and No. 5,  $3\frac{1}{2}$ ,  $9\frac{1}{2}$  and  $16\frac{1}{2}$  inches respectively above the rear teat. Before the histological studies were completed some evaporation of the preserving solution occurred and blocks Nos. 4 and 5 were spoiled. Slides were made from blocks 1, 2 and 3, of which representative areas are shown in Fig. 5, A, B & C. The structure of the edematous tissue obtained from one of the frozen gross sections and photographed at the same magnification as the other histological areas, is shown in Fig. 5, D.



FIG. 3. Gross structure of udder tissue in vertical transverse plane through the rear teat, showing thick layer of edematous swelling along the base between the gland tissue and the skin. Note how the teat appears to be shortened.

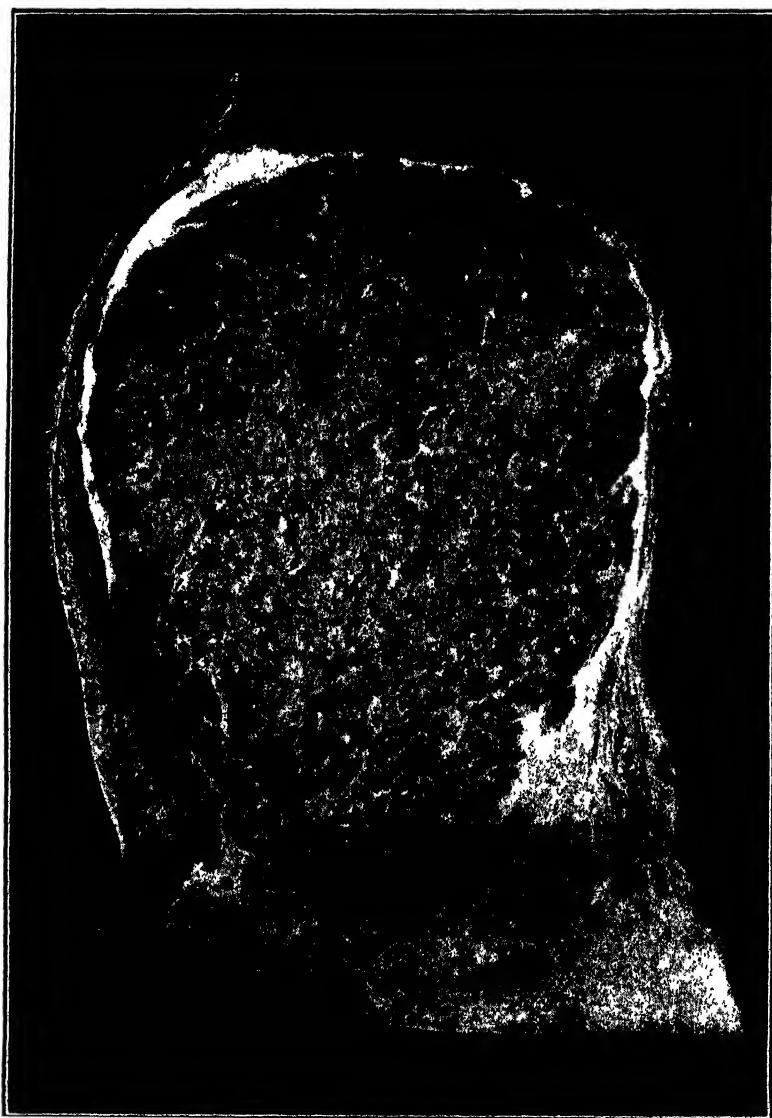


FIG. 4. Gross structure of udder tissue in a vertical transverse plane through a front teat. Although this surface was photographed more than 12 months after the udder was sectioned, the appearance is essentially unchanged.

Although the appearance of areas A and C which were taken from locations only a short distance above the front and rear teats differs somewhat from area B, which was located not far from the abdominal attachment of the front quarter where the tissue might be expected to be more actively secreting, these areas are entirely different in structure from area D which was taken from the edematous tissue. Apparently the edematous swelling does not invade the glandular tissue to any appreciable extent but is confined chiefly to the space between the mammary gland and the skin.

A part of the routine that is followed in connection with the post-mortem studies of the cow udders at the Beltsville Station involves a number of tests of the tissue after it has been filled with formalin, frozen, and cut into gross sections approximately one inch in thickness. Discs having an area of 6.25 square inches, are cut from certain gross sections at definite locations. Among the several determinations made are tests of the sponginess of the tissue of different udders and of the tissue from different parts of the same udder. The discs are soaked in water under vacuum, weighed, subjected to a pressure of 312.5 pounds to eliminate most of the water and weighed again. They are then resoaked as before and reweighed after which the degree of recovery and the amount of fluid taken up and held by the tissues per unit of pressed weight are determined.

After pressing and resoaking the weight of the discs taken from the glandular tissues of the udder from cow 848, averaged 97.7 per cent and those from the edematous tissue averaged 96.6 per cent of the soaked weight before the pressure was applied, indicating a very high recovery. The edematous tissue, however, was decidedly more spongy than the glandular tissue of this udder as it took up a quantity of water equal to 4.41 times its pressed weight as compared with an average of 1.91 times its pressed weight for the glandular tissue from a number of different locations. The pressed edematous tissue was particularly dry in appearance and seemed to consist almost entirely of loosely connected sheets or layers of light colored connective tissue running nearly parallel to each other. Air-dried edematous tissue was found to be only 41 per cent as heavy as air-dried glandular tissue. The air-dried weight was only 7 per cent of the weight before pressing for the edematous as compared with 16 per cent for the glandular tissue.

There has long been a question as to whether or not intense swelling of the udder at calving time is objectionable. Observations of A. G. Van Horn, Superintendent at the Woodward, Oklahoma, Station of the Bureau of Dairy Industry, indicate that the daughters of one sire used at that Station were particularly subject to intense and persistent swelling and that the swelling prevented a number of animals from reaching their maximum producing ability. Apparently the swelling described by Van Horn was edematous in character and similar to the condition described in connection with cow No. 848. Frequently it was accompanied by abdominal swelling, especially in

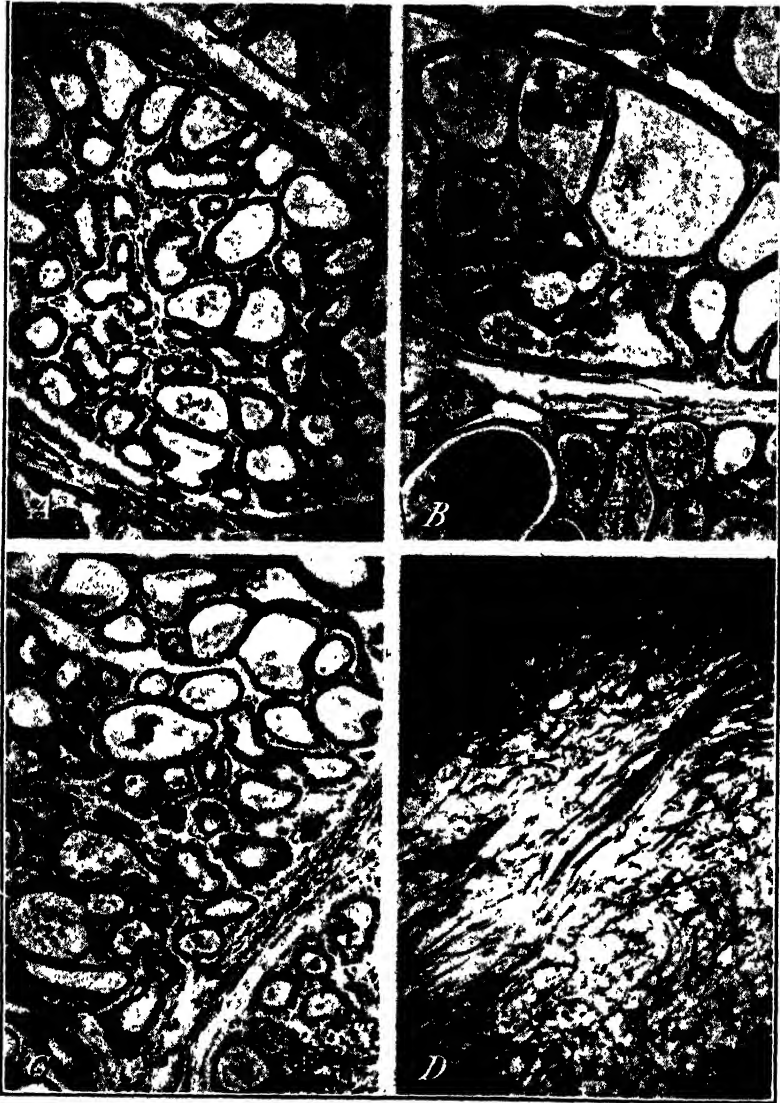


FIG. 5. Photomicrographs of mammary gland and edematous tissue: A, gland tissue from lower portion of front quarter; B, gland tissue from upper portion of front quarter; C, gland tissue from lower portion of rear quarter; D, edematous tissue.

young cows. It was reported that the swelling remained longest in the poorest attached udders.

As previously indicated cow No. 848 habitually showed edematous swelling at each parturition. Photographs taken 5 days before second calving, 7 days after third calving and 1 day after fourth calving give some idea of the extent to which it occurred. The one taken before second calving shows not

only considerable udder swelling but a definite abdominal swelling in the region of the navel. (Fig. 2.)

In production, cow No. 848 was somewhat erratic. During the first lactation which commenced at 2 years 1 mo., she reacted to the agglutination test (Bang's disease) and was moved to other quarters but was continued on the same schedule of feeding and milking and did not appear to suffer any marked reduction in milk flow. Her production, however, was low—never going above 42.5 pounds on any day—and amounted to only 5698 pounds of milk and 187 pounds of butterfat in 365 days. Calving again at 3 years 3 mos. of age she reached a high point of 80.2 pounds of milk in one day and produced a total of 11,695 pounds of milk and 377 pounds of butterfat in 323 days. In her third lactation, at 4 years 5 months, her highest production for a single day was 74.0 pounds of milk and her total for 365 days was 15,200 pounds of milk and 520 pounds of butterfat.

Routine studies of the rate of milking with a milking machine indicated that she was a slow milker. Timing studies were carried on during the first, second and third lactations. At the first 2-day timing, 121 days after first calving she was producing an average of 22.8 pounds of milk daily. The time required for milking was 8.35 minutes, 31.4 per cent of which was spent in massaging, and 20.8 per cent of the total yield of milk was obtained during the massaging period. At the second 2-day timing, which occurred 119 days after second calving, her average production was 42.25 pounds of milk daily, the time required for milking was 9.75 minutes of which 31.2 per cent was spent in massaging the udder, and 38.3 per cent of the total yield was obtained during the massaging period. The third timing occurred 49 days after third calving, when she was producing an average of 71.75 pounds of milk daily. This time 13.10 minutes was required for milking. Only 2.8 per cent of that time was spent in massaging and the massaging yielded only 0.6 per cent of the total quantity of milk.

#### CONCLUSIONS

Aside from the edematous swelling there was nothing about the appearance of the udder of cow No. 848 at any time to indicate any significant abnormality and all four quarters of the udder were functioning at the time of slaughter. The point that stands out as of particular interest is the fact that the intense swelling, which occurred in the udder of this cow at calving time apparently was edematous in nature, and that it did not appear to invade or affect the secreting tissue to any appreciable extent, but on the contrary was confined chiefly to the space between the glandular tissue and the skin.



# THE BREEDING EFFICIENCY OF PROVED (AGED) SIRES

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How many years of usefulness can be expected from a dairy sire that is 5 years of age or older? How frequently can such a sire be used successfully? Do seasonal changes have any effect on the breeding efficiency or fertility of aged sires? What effect if any does moving, and the subsequent change in environment, have on the usefulness of an old sire?

The Bureau of Dairy Industry has not only used proved sires in its various experimental station herds for a number of years, but it has probably used more proved sires than any other institution. Recognizing that the records of these sires might disclose some information with which to answer these questions, the author has analyzed the service records of 20 proved sires used in the station herds. The results of the study are reported in the following pages.

## SOURCE AND NATURE OF THE DATA STUDIED

The data for this study were collected from the breeding records of the Bureau's experimental herds at Jeanerette, La., Lewisburg, Tenn., Hannibal, Mo.,<sup>1</sup> Columbia, S. C.,<sup>1</sup> Huntley, Mont., Mandan, N. Dak., Woodward, Okla.,<sup>1</sup> and Ardmore, S. D.<sup>2</sup>

The study includes the breeding records of 20 proved sires for which the data were complete and known to be accurate. Other proved sires were used to a limited extent in these herds, or for short periods, and they will be referred to in the discussion.

The data do not afford a comparison between young and old sires because very few young sires have been used and only for short periods. Neither was it possible to determine the breeding condition of the females in these herds, except in a general way. All the herds were managed by experienced men, most of whom were capable of treating ordinary breeding troubles. Veterinarians were employed for the most difficult cases, and it is believed that breeding trouble with females was no more serious in these herds than in the average breeding herd. The herds were subjected periodically to tests for Bang's disease. There was an occasional female reactor in some of the herds. None of the bulls included ever reacted to the Bang's test.

In this study, the breeding efficiency or fertility of a sire, as indicated by the number of services per conception when the sires were mated to fertile females, is expressed both by ratios and by percentages.

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<sup>1</sup> Dairy work at these stations is in cooperation with State agricultural experiment stations.

<sup>2</sup> Dairy work at this station was discontinued in 1932.

The data were tabulated and arranged to show breeding efficiency or fertility, by months, by years, and by ages, for the 20 sires during the time they remained fertile and in service in the station herds. The services to all cows include those to fertile cows, sterile cows, and to cows in which pregnancy was doubtful, and are of interest in showing whether there is any relation between frequency of service and breeding efficiency.

For the purpose of this study a female was considered fertile up to the time of her last conception, no matter how many times she was bred for her last conception or to how many sires. All services to a cow, after her last conception, were credited to the bulls as services to a sterile female unless it was definitely known that the cow was pregnant.

In a few cases, sires were continued in service for some time after they became sterile because the fact that they were sterile was not evident at the time. All services credited to a sire, after he got his last conception are omitted from the comparative study. Only services occurring after a sire was 5 years of age are included in the tabulation.

### *Sires Studied*

For convenience in presenting the data, the 20 sires have been numbered from 1 to 20. Table 1 gives the breed of each sire, his age when he began service in the station herd, his age when his use was actually discontinued, his age when he was judged infertile, and the length of his fertile service in the station herd.

The 20 sires include 8 Jerseys, 2 Guernseys, and 10 Holsteins. With the exception of sires 9, 11 and 14, all these bulls were proved sires when they began service in these station herds, and they varied from 5 to 10 years of age at the time. Sires 9, 11 and 14 were started in service as young unproved bulls, but were proved later.

### FEEDING AND MANAGEMENT OF THE BULLS

All the bulls had been moved to the station at which they were used shortly before the start of their service. Bulls 3, 5 and 6 were moved rather short distances by truck, but the others were shipped several hundred miles by train. This movement was necessarily accompanied in most cases by abrupt changes in environmental and climatic conditions.

Attention is directed to the wide range of climatic conditions at the stations where these bulls were in service, as this may have a bearing on some of the points discussed later.

All the bulls were kept in strong pens and were allowed to run in or out of a shed at will. The rations varied somewhat according to the station. A good quality of legume hay was fed, but was given in limited amounts if the bull was inclined to develop a large middle. Grain, usually the mixture fed to the regular herd, was fed in sufficient amounts to keep the bulls in

TABLE 1

*Age of the 20 sires when they started service in the Bureau's station herds, age when their use was discontinued, age when they were judged infertile, and the length of their fertile service in these herds after they were 5 years old or older*

Sire No.	Breed	Age when service began in these herds	Age when use was discontinued	Age when sire was judged infertile	Length of fertile service in the herd, after 5 years of age	Station where sire was used
		Yr.-Mo.	Yr.-Mo.	Yr.-Mo.	Yr.-Mo.	
1	Jersey	5-11	10-9	10-9	4-10	Jeanerette, La.
2	Jersey	8-3	13-5	13-3	5-0	Jeanerette, La.
3	Jersey	7-1	(1)	(1)	(1)	Hannibal, Mo.
4	Jersey	10-5	15-2	14-6	4-1	Lewisburg, Tenn.
5	Guernsey	10-3	13-3	13-3	3-0	Columbia, S. C.
6	Guernsey	7-3	13-10	12-0	4-9	Columbia, S. C.
7	Holstein	6-10	12-3	12-3	5-5	Huntley, Mont.
8	Holstein	5-5	10-7	(2)	5-2	Mandan, N. Dak.
9	Holstein	4-4	8-0	(2)	3-0	Huntley, Mont.
10	Holstein	6-2	11-4	11-2	5-0	Mandan, N. Dak., and Huntley, Mont.
11	Holstein	1-5	6-10	(2)	1-10	Woodward, Okla.
12	Holstein	6-0	12-5	12-5	6-5	Woodward, Okla.
13	Holstein	5-4	9-11	(2)	4-7	Woodward, Okla.
14	Holstein	3-8	16-1	16-1	11-1	Huntley, Mont., and Ardmore, S. D.
15	Jersey	7-2	8-9	8-9	1-7	Jeanerette, La.
16	Jersey	7-9	10-0	10-0	2-3	Jeanerette, La.
17	Jersey	7-9	9-10	9-10	2-1	Jeanerette, La.
18	Jersey	7-11	12-0	12-0	4-1	Jeanerette, La.
19	Holstein	6-8	(1)	(1)	(1)	Woodward, Okla.
20	Holstein	6-11	(1)	(1)	(1)	Mandan, N. Dak.
Average		6-8	10-10 <sup>3</sup>	12-0 <sup>4</sup>	4-5 <sup>3</sup>	

<sup>1</sup> Still fertile and in active service as of May 31, 1937.

<sup>2</sup> Fertile when disposed of.

<sup>3</sup> Average for 17 sires.

<sup>4</sup> Average for 13 sires.

fair condition. Silage was fed sparingly. The bulls were encouraged to exercise but no systematic plan of exercising was followed.

#### FEEDING AND MANAGEMENT OF THE COWS

The females in the various herds where the bulls were in service were fed and managed under desirable conditions. All cows on official test were given a good grade of legume hay and silage, and a grain mixture. As a rule, the cows on test received no pasture. The regular herd cows were fed legume hay, silage, and a limited-grain ration, and pasture. Many of the cows were in feeding experiments in which they received only roughage of good quality. Studies by the Bureau indicate that this practice does not affect the fertility or breeding condition of cows.<sup>5</sup> Pasturage at the stations varied in kind and quality, with the region and climatic conditions.

<sup>5</sup> Graves, R. R., and Dawson, J. R. Feeding Dairy Cows on Alfalfa Hay Alone. U. S. Dept. Agr. Tech. Bul. 610, 1938.

When cows were on test they were bred approximately 5 months after calving, whereas cows in the regular herd were bred 2 to 3 months after calving. Heifers were bred for the first time at approximately 15 months of age.

#### RESULTS AND DISCUSSION

##### *Length of Fertile Service in the Herds*

According to the data in Table 1, the 20 sires were started in service in these herds at the average age of 6 years 8 months. Eliminating sires 9 and 11, which were started in service as young bulls, the average age that the remaining 18 sires were started in service was 7 years, 1 month. Three of the sires are still in service and the average age of the other 17 was 10 years 10 months when their use was discontinued. Thirteen of the 20 sires died or were infertile at the time they were disposed of at an average age of 12 years. The average period of fertile service for sires in these herds was 4 years 5 months for services after the age of 5 years. Only 17 of the 20 sires were included in this average since sires 3, 19, and 20 are still in service.

In addition to the sires shown in Table 1, other proved sires were used in the station herds. Seven of these were victims of injury or disease after a short period of service, and two were frequently shifted from one herd to another so that the breeding data on these sires are not considered suitable to use in this study. The shortest period of service by any of the 29 sires was 8 months and the longest period of fertile service was 11 years 1 month by sire 14, not including his services prior to 5 years of age.

Sire 14 was fertile up to the time of his death at 16 years 1 month of age. A sire in the Bureau's herd at Beltsville, Md., was fertile to the age of 16 years 4 months. A sire in South Carolina<sup>4</sup> is reported as being in service and fertile at 17 years 1 month of age.

##### *Fertility of the Sires*

Table 2 shows the number of services to all cows, by each sire during the time he was used in the station herd, also the number of services to fertile cows only, and the number of conceptions obtained. The fertility of the sire is represented by the percentage of services to fertile cows that resulted in conceptions.

The 20 sires had a total of 3,585 services to all cows, of which 2,982 were to fertile cows. The total number of conceptions was 1,197. This is a ratio of 2.49 per conception, based on services to fertile cows.

The 20 sires varied in fertility, from a low of 21 per cent for sire 4 to a high of 71 per cent for sire 16. The average for the 20 sires was 40 per cent, and 10 of the sires were below the average for the group.

<sup>4</sup> Fern's Raider of Appin 64700, a Guernsey sire owned by C. S. McCall, Bennettsville, S. C. Information to the author through J. P. LaMaster, Clemson College, S. C.

TABLE 2

*The relative fertility or breeding efficiency of the 20 sires during the time they were used in the station herds*  
(Only services after 5 years of age are included)

Sire no.	Services to all cows	Services to fertile cows	Conceptions	Breeding efficiency, <sup>1</sup> or fertility
	No.	No.	No.	Per cent
1	326	254	78	31
2	121	72	19	26
3	160	144	58	40
4	267	235	50	21
5	135	120	35	29
6	147	133	49	37
7	181	170	57	34
8	141	117	72	62
9	142	126	41	33
10	397	356	144	41
11	87	63	29	46
12	196	166	109	66
13	166	146	96	66
14	159	145	72	50
15	97	65	23	35
16	58	44	31	71
17	98	71	40	56
18	292	211	47	22
19	251	209	53	25
20	164	135	94	70
Total	3,585	2,982	1,197	...
Average	179	149	60	40

<sup>1</sup> Based on the number of services to fertile cows that resulted in conceptions.

Miller and Graves<sup>5</sup> reported the breeding records of 10 mature bulls and 18 young bulls used at the Beltsville, Md., station. The 10 mature bulls had 1,109 services to fertile females and got 289 conceptions, a ratio of 3.83 services per conception.

The ratios of services per conception, both in this study and in the Beltsville report, seem very high. The herds all have had breeding trouble at one time or another, as a result of infections of various kinds. These high ratios are undoubtedly due in part to the use of older sires and also to the fact that sterile cows are held longer in these herds, in an effort to get them to breed, than would be the case in most herds.

### *Effect of Age on Fertility*

Table 3 gives the number of services to fertile cows, the number of conceptions, and percentage of services that resulted in conceptions, for the 20 sires according to age by 6-month periods starting at 5 years of age.

<sup>5</sup> Miller, Fred W., and Graves, R. R. Reproduction and Health Records of the Beltsville herd of the Bureau of Dairy Industry. U. S. Dept. Agr. Tech. Bul. 321. 1932.

TABLE 3  
*The effect of advancing age on fertility of sires*

Age	Sires included	Services to fertile cows	Conceptions	Fertility <sup>1</sup>
Years	No.	No.	No.	Per cent
6-month period				
5 to 5½ .....	5	46	24	52.2
5½ to 6 .....	6	67	33	49.3
6 to 6½ .....	8	100	52	52.0
6½ to 7 .....	10	132	69	52.3
7 to 7½ .....	12	220	89	40.5
7½ to 8 .....	14	256	115	44.9
8 to 8½ .....	14	256	121	47.3
8½ to 9 .....	15	325	144	44.3
9 to 9½ .....	15	324	131	40.4
9½ to 10 .....	14	325	122	37.5
10 to 10½ .....	13	219	74	33.8
10½ to 11 .....	13	225	57	25.3
11 to 11½ .....	10	122	49	40.2
11½ to 12 .....	8	83	31	37.3
12 to 12½ .....	7	64	20	31.3
12½ to 13 .....	3	47	19	40.4
13 to 13½ .....	4	89	20	22.5
13½ to 14 .....	2	31	12	38.7
14 to 14½ .....	2	31	8	25.8
14½ to 15 .....	2	8	5	62.5
15 to 15½ .....	...	...	...	...
15½ to 16 .....	1	9	3	33.3
16 to 16½ .....	1	3	1	33.3
2-year periods				
5 to 7 .....	11	345	178	51.9
7 to 9 .....	16	1057	469	44.4
9 to 11 .....	18	1093	384	35.1
11 to 13 .....	9	316	119	37.7
13 and over—	4	159	45	28.3

<sup>1</sup> Based on number of services to fertile cows that resulted in conceptions.

It is of interest to mention the extreme variation in fertility of the individual sires as age advanced. Sire 1, as one example, was 100 per cent fertile at the age of 5½ to 6 years, and his fertility dropped to 70 per cent during the following 6-month period. At the age of 6½ to 7 years, however, he got only 1 conception in 15 services to fertile cows, indicating a fertility of only 6.7 per cent. His fertility reached 51.9 per cent at the age of 8 to 8½ years, then dropped sharply to 19.1 per cent. During the 6-month period of his heaviest service, from 9½ to 10 years, his fertility was 32.6 per cent, when he got 15 conceptions out of 46 services. For the next 6-month period he got only 1 conception out of 34 services, indicating a fertility of only 2.9 per cent.

Sires 8, 12, 13, 16, and 20 for the most part had a high fertility throughout the years they were used.

Sire 14 remained fertile to the oldest age of any of the 20 sires. While there were a few periods when his fertility was low, he showed a high degree of fertility throughout most of his life and up to the time of his death at 16 years 1 month of age.

Table 3 also shows the relation of fertility to advancing age, by 2-year age periods. At 5 to 7 years of age, the fertility of the group was 51.9 per cent, decreasing to 44.4 per cent at 7 to 9 years of age, 35.1 per cent at 9 to 11 years of age, slightly increasing to 37.7 per cent at 11 to 13 years of age; with a further steady decrease to 28.3 per cent for services after 13 years of age. On the average there was a decided and consistent decline in fertility with advancing age, but the breeding records of the individual sires indicate that there is great variation in this respect and that conclusions based on averages should be carefully considered.

TABLE 4

*The effect of frequency of service on fertility*

Frequency of service (Services per sire per month)	Services to all cows				Services to fertile cows only			
	Number of sires included	Services	Conceptions		Number of sires included	Services	Conceptions	
No.	No.	No.	No.	Per cent	No.	No.	No.	Per cent
1	20	156	63	40.4	19	169	97	57.4
2	19	326	135	41.4	20	340	160	47.1
3	19	360	137	38.0	20	363	155	42.7
4	20	408	130	31.9	19	420	161	38.0
5	19	495	166	33.5	20	395	158	40.0
6	18	330	132	40.0	17	324	149	46.0
7	17	329	109	33.1	17	240	92	38.3
8	15	296	89	30.1	11	248	60	24.2
9	15	315	78	24.8	8	171	62	36.3
10	5	110	28	25.5	3	40	11	27.5
11	9	132	39	29.5	6	88	32	36.3
12	5	84	19	22.6	2	36	12	33.3
13	4	52	28	53.8	2	26	16	49.5
14	2	28	9	32.2	2	42	11	26.2
15	3	90	18	20.0	3	45	17	37.8
16	2	32	13	40.6	1	16	2	18.8
17	..	..	..	..	..	..	..	..
18	..	..	..	..	..	..	..	..
19	1	19	2	10.5	1	19	2	10.5
20	..	..	..	..	..	..	..	..
21	..	..	..	..	..	..	..	..
22	..	..	..	..	..	..	..	..
23	1	23	2	8.7	..	..	..	..
1 to 3, inc.	20	842	335	39.8	20	872	412	47.2
4 to 6, inc.	20	1233	428	34.7	20	1139	468	41.1
7 to 9, inc.	20	940	276	29.4	20	659	214	31.0
10 or more...	14	570	158	27.7	8	312	103	33.0

*Effect of Frequency of Service on Fertility*

What effect does frequency of service have on the fertility of old sires?

Table 4 shows the number of services, to all cows and to fertile cows, the number of conceptions, and the fertility of the 20 sires, arranged according to the number of services per calendar month. Considering services to fertile cows only, the trend was decidedly toward a lower fertility as the number of services increased from 1 to 8 per month. As the number increased beyond 8, there did not appear to be any further decline in fertility. However, many of the sires showed just as high or higher fertility when used from 7 to 15 times a month as they did when used only once a month.

It is probable that the most pronounced effect of frequency of service on fertility will not be felt until a time following the period of service. With this point in mind, the data were tabulated by months and by sires according to the number of total services that particular sire had had during the preceding month. Table 5 gives the results of this tabulation.

TABLE 5

*The effect of frequency of service in one month on the fertility of the sires during the following month*

Services during preceding month	Number of sires included	Services to fertile cows, conceptions, and fertility for the following month		
		Services	Conceptions	
No.	No.	No.	No.	Per cent
No services . . . . .	16	91	43	47.3
1 to 3, inclusive . . . . .	20	949	423	44.6
4 to 6, inclusive . . . . .	19	896	365	40.7
7 to 9, inclusive . . . . .	18	666	230	34.5
10 and over . . . . .	12	304	90	29.6

Sixteen sires had 91 services and secured 43 conceptions, indicating 47.3 per cent fertility, during months that followed months when no services were permitted. When 1 to 3 services were permitted per month, the fertility for the following month was 44.6 per cent. The decline in fertility on this basis of interpretation is rather rapid and consistent. When 10 or more services were permitted in one month, the fertility for the following month was 29.6 per cent. It would appear that a rest period of a month is of distinct benefit, and that as the number of services per month increases to 10 or more there is a decided and consistent drop in fertility the following month. Here again, however, was found great variation between individual sires.

It is a matter of interest to note that 24 per cent of all services to the 20 sires studied were at the rate of 1 to 3 per month; 35 per cent were at the rate of 4 to 6 per month; 26 per cent were at the rate of 7 to 9 per month; while only 16 per cent of all services were at the rate of 10 or more per month. One sire was used 23 times, during one month, and another sire was used 19 times.

*Effect of Season on Fertility*

Are old bulls more fertile at some seasons of the year than at others? Table 6 shows the number of services to all cows and to fertile cows, the conceptions, and the fertility of the 20 sires according to the month of the year in which the services were performed.

TABLE 6  
*The effect of season on fertility of sires*

Month in which services occurred	Services to all cows	Services to fertile cows only	Conceptions	Breeding efficiency or fertility <sup>1</sup>
	<i>No.</i>	<i>No.</i>	<i>No.</i>	<i>Per cent</i>
January	291	235	94	40.0
February	263	227	97	42.7
March	311	262	107	40.8
April	323	262	116	44.3
May	340	279	110	39.4
June	281	237	90	38.0
July	310	266	115	43.2
August	281	233	94	40.3
September	251	205	80	39.0
October	313	261	108	41.4
November	307	262	85	32.4
December	314	253	101	39.9

<sup>1</sup> Based on services to fertile cows that resulted in conceptions.

There is a tendency for the fertility to be somewhat higher during the months of February, April, July, and October, when the fertility (based on services to fertile cows) averaged 42.9 per cent. The low trends come in June, September, and November, during which months the average fertility was 36.5 per cent. This difference of 6 per cent is probably of little significance, however, when one considers the wide range of climatic conditions obtaining at the stations where the sires were used (see Figure 1).

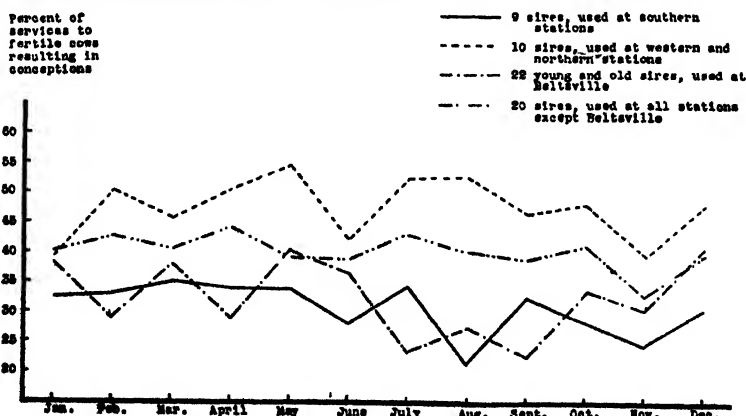


FIG. 1. Effect of season of the year on fertility of sires.

Miller and Graves,<sup>6</sup> in a study of 22 young and old bulls used only at the Beltsville, Md., station covering a period of 4 years and including 1,539 services to fertile cows, found the lowest fertility (23 per cent) in July and September, after which the fertility increased during the fall and winter months (see Figure 1). They suggested that the hot weather influences the functioning of the genital organs adversely and that increased genital efficiency seems to have been associated with the advent of autumn. This apparently was not the case when all of the 20 bulls included in this study are considered.

Because of the wide range of climatic conditions at the several stations, 19 of the sires were divided into two groups according to whether they were in service at a southern station or at western and northern stations. Nine sires (1, 2, 4, 5, 6, 15, 16, 17, and 18) were placed in the southern group, and 10 sires (7, 8, 9, 10, 11, 12, 13, 14, 19, and 20) in the western and northern group.

The fertility curves for the two groups follow the same general trend (see Figure 1), but the curve for the southern group follows that of the Beltsville group more closely than does the curve of the western and northern group. Climatic conditions at Beltsville are also more similar to those of the southern group of stations.

It is of further interest to note the decidedly lower range in fertility of the sires used at the southern stations as compared to those used at the western and northern stations. Those used at the southern stations had an average fertility of 36 per cent, while those used at the western and northern stations averaged 49 per cent. Higher temperatures during the summer with higher humidity generally prevail at the southern stations. At the western and northern stations during the summer the temperature rises quite high, but as a rule drops sharply at night. Lower humidity prevails at the western and northern stations than at the southern stations.

### *Effect of Moving on Fertility*

Does the moving of sires for a considerable distance with the accompanying change in environmental conditions lower the fertility of old bulls and, if so, how long does this condition last?

Only 17 of the 20 sires are considered suitable for this phase of the study, because they were transported for considerable distances before their services as proved sires in the station herds began. Unfortunately, there is no accurate record of the fertility of most of them before they were moved. Table 7 shows the approximate distance in miles that the sires were transported, and the fertility by 3-month periods for 2 years following their initial service in the herds.

<sup>6</sup> *Loc. cit.*

TABLE 7

*The effect of shipment and changes in environment on the fertility of 17 sires, as indicated at stated periods during the first and second year after arrival at the respective stations<sup>1</sup>*

Sire number	Distance moved	Fertility of sires during first year									
		1 to 3 months		4 to 6 months		7 to 9 months		10 to 12 months		Ser- vices	Conceptions
		Ser- vices	Conceptions	Ser- vices	Conceptions	Ser- vices	Conceptions	Ser- vices	Conceptions		
	Miles	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
1	1,100	8	100.0	4	1	25.0	1	50.0	8	0	00.0
2	200	10	30.0	5	4	80.0	4	50.0	6	2	33.3
3	125	8	50.0	11	4	36.4	5	45.5	13	6	46.2
4	630	30	26.7	30	6	20.0	3	21.4	16	2	12.5
5	85	7	0.0	9	1	11.1	2	13.3	6	3	50.0
7	685	3	0.0	6	1	16.7	1	14.3	19	5	26.3
8	370	2	50.0	3	1	33.3	0	0.0	7	4	57.1
10	370	13	53.8	17	3	17.6	8	47.1	22	8	36.4
12	815	8	75.0	13	7	53.8	11	81.8	2	0	00.0
13	600	3	66.7	10	6	60.0	7	58.3	10	5	50.0
14	300	4	75.0	6	4	66.7	2	100.0			
15	1,100	2	50.0	10	4	40.0	3	20.0	11	2	18.2
16	835	1	100.0	5	2	40.0			2	2	100.0
17	1,100	6	66.7	2	2	100.0	11	63.6	18	9	50.0
18	1,785	17	5.9	14	3	21.4	9	11.1	11	3	27.3
19	815	21	14.2	22	4	18.2	10	40.0	33	3	9.1
20	370	14	92.9	5	5	100.0	7	57.1	11	8	72.7
Total		157	41.4	172	58	33.7	61	40.1	195	62	31.8

<sup>1</sup> All sires were transported in freight cars, except Nos. 3, 5, and 6, which were moved by truck.



For the first 3 months following arrival at the stations the 17 sires had an average fertility of 41.4 per cent, which is remarkable because of the decline to 33.7 per cent at 4 to 6 months following arrival. The high average fertility immediately following shipment is unexplainable unless the time was too brief for the full effects of shipping and changes in environment to be exerted. Or possibly the change of environment may have had a temporary stimulating effect on fertility.

The lowest point in fertility (31.8 per cent) was 10 to 12 months after the bulls arrived, after which there was a decided trend toward higher fertility during the second 12 months of service in spite of the advancing age of the sires. The average fertility for the first year following movement of the sires was 36.4 per cent as compared to 40.7 per cent for the second year.

The individual sires offer interesting studies. Sire 1 showed 100 per cent fertility for the first 3 months following arrival, which was the highest for the 5 years he was used. He was moved 1,100 miles from Beltsville, Md., to Jeanerette, La., with a very decided change in climatic and other environmental conditions. Sires 5 and 7 got no conceptions for the first 3 months, but their fertility increased gradually from then on. Sire 5 was moved only 85 miles by truck, and there was practically no change in climatic conditions. While the average fertility is decidedly downward for the first year following movement of the sires and is decidedly upward for the second year, the individual variation is so wide that definite conclusions cannot be drawn.

#### SUMMARY AND CONCLUSIONS

Detailed service records of 20 proved sires used in 8 branch experiment station dairy herds are presented and the fertility of these sires after 5 years of age is expressed by the percentage of services to fertile cows that resulted in conceptions. Extra services and services to infertile cows are given separately. The data were tabulated and analyzed from the standpoint of (1) the relative fertility of the individual sires, (2) the effect of advancing age on fertility, (3) the effect of frequency of service on fertility, (4) the effect of season of the year on fertility, and (5) the effect of moving sires on their fertility.

Because of the extreme and inconsistent variation in fertility exhibited by individual sires on all phases included in this study, it is apparent that averages are of little value for application to individual sires.



## SOFT CURD MILK\*

### *A Critical Review of the Literature*

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When milk from individual cows is coagulated with rennin and (or) pepsin, the toughness and adhesiveness of the coagulum varies widely with different animals. Some milk forms a soft, friable type of curd which might be described as "mushy." Other milk exhibits an extremely tough rubbery curd mass which fractures with difficulty. Milk with the former character of coagulum has come to be known as soft curd milk while that with the latter type of coagulum is known as hard curd milk.

The interest in soft curd milk has been increasing steadily since 1923 when Hill (35) proposed his test for determining the curd character of milk as an index to its suitability for infant feeding. Previous to this time Washburn and Bigelow (81), Washburn and Jones (82), Buckley (9) and Alleman and Schmid (1) had pointed out marked differences in the coagula obtained with different milks when treated with digestive enzymes but, with the exception of Alleman and Schmid, none of these workers developed a means of accurately classifying the curd characteristics and their work was largely overlooked.

As early as 1913 Brennemann (6) explained the favorable results obtained with boiled milk in infant feeding on a curd character basis and intimated that other successful modifications of cow's milk for infant use depend primarily on the ability of the modifier to alter the physical properties of the coagulum (particularly the particle size) formed in the stomach after ingestion.

The interest of the milk distributors in soft curd milk has grown tremendously in the past few years and due to the fact that the use of evaporated milk in infant feeding has been increasing rapidly (largely at the expense of fluid milk), it is their desire to place on the market a fresh milk having digestion characteristics as suitable for infants as has the canned variety. They have been more interested in the subject since it has been found possible to modify or soften the curd character of average mixed milk by processing rather than having to resort to a selection of individual cows giving milk with the desirable characteristics, as originally suggested by Hill (36, 37, 39) and as practiced by dealers in several cities where soft curd milk has been sold for the past 10 years.

That the medical profession is alert to the possibilities of soft curd milk is evidenced by the approval of "Soft Curd Certified Milk" by the Amer-

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ican Association of Medical Milk Commissions and its endorsement by physicians and pediatricians in many places; also by the recent action of the Council on Foods of the A. M. A. in publishing a report concerning the nutritional significance of the curd tension of milk (18).

The purpose of this article is to review the subject of soft curd milk, to summarize the available information and to criticise it in a constructive manner.

#### THE CURD TEST

Hill's original test (35) for measuring the curd tension or curd toughness of milk has been criticised and modified by a number of workers (10, 15, 48, 58, 59) as well as by himself (9). Recently a committee of the American Dairy Science Association, after two years of study, has attempted to standardize the determination by reporting a tentative method (19). Briefly, this method substitutes N/10 hydrochloric acid, containing 0.45 per cent pepsin for the calcium chloride pepsin reagent originally used; adds tempered milk to the tempered coagulant in a 250 ml. beaker or 8 oz. mayonnaise jar; establishes  $95^{\circ}\text{F.} \pm 1^{\circ}\text{F.}$  as the coagulation temperature and 10 minutes as the coagulation interval; requires the addition of the 100 ml. charge of milk to the 10 ml. of reagent by means of a 100 ml. pipette with the tip removed so that it drains water in  $4\frac{1}{2}$  seconds, the milk to be added holding the pipette vertically over the center of the receptacle and blowing it into the coagulant in approximately 2 seconds with no further agitation; requires a knife of similar design to that used in the American Curd-O-meter with the same linear cutting surface; specifies that any accurate instrument may be used which embodies an automatic movement either of the knife or receptacle, provided that the rate be approximately one inch in five seconds; and demands that the result be the average of two or more maximum readings checking within 10 per cent. If the test is carried out as described the pH of the whey from the cut samples will be between 5.95 and 6.10 depending on the buffer capacity of the milk.

It is the hope of the committee that this tentative method will eliminate the diversity of procedures in use the last few years and make for uniformity. Undoubtedly some of the discrepancies in the literature are due to the wide differences in methods used.

The curd tension instrument in use to the greatest extent at present is the American Curd-O-meter made by Heusser Instrument Company of Salt Lake City. This equipment has been found satisfactory if an automatic method of raising the curd receptacle against the knife is provided. The simplest means of accomplishing this is through the use of a small hydraulic lift which can be constructed easily by any sheet metal worker.

In 1936 the Submarine Signal Company, of Boston, developed an instrument with a knife similar to that employed by the Curd-O-meter except that it is suspended from a gauge floating in a column of mercury and moves

downward through the curd at an even rate, actuated by a motor. When properly used this instrument and the Curd-O-meter give closely agreeing results.

Within the last year, Chambers and Wolman (14, 87) have described a method of determining curd particle size when milk is coagulated in "artificial stomachs" under conditions arranged to simulate those in vivo. Their method calculates the curd surface of the coagulated milk as an index to its digestibility. This method, providing the conditions are what they should be, would seem to offer a more accurate gauge of the digestion characteristics of milk than curd tension, since the rate of gastric digestion and the rate of stomach clearance appear to be related to curd surface area (7, 22) or curd particle size (6, 26) rather than to curd toughness as such. More study is needed before this idea is accepted and perhaps some modifications and simplifications of the suggested technique would be desirable before the method is offered as being superior to that of curd tension. Chambers and Wolman find a general relationship between their "curd numbers" and curd tension as determined by the original Hill method. There are, however, some rather wide discrepancies in the case of certain modified milks.

#### NATURAL SOFT CURD MILK

*Composition and Properties.*—Normal, individual, cow's milk will vary in curd tension from about 15 grams to about 150 grams by the Hill method. Using the tentative method (19) or Miller's procedure (58), the range will be wider, soft curd milk giving about the same results but hard curd milk showing higher values (24, 58). While Hill originally suggested an upper limit of 20 grams (37) for soft curd milk, at present by common consent, milk with a tension under 30 grams (in a few cases 33 grams) is considered as soft curd.

Natural soft curd milk is found in all breeds of dairy cattle but predominates in the Holsteins, followed in order by the Ayrshires, Brown Swiss, Guernseys and Jerseys (2, 5, 22, 37, 39, 66). It is invariably low in total solids, solids-not-fat (particularly casein), fat, and probably ash (22, 26, 38, 59, 60, 83). Several studies have failed to indicate that there is any inherent qualitative difference between soft curd milk and hard curd milk or between the respective ingredient substances, the work of Weisberg *et al.* (83) being outstanding. It appears, therefore, that soft curd milk is not a different kind of milk but merely a milk low in solids and of high water content. The ingredient of milk most closely related to curd tension is casein (2, 22, 83). Doan and Welch (22) assert that curd tension is a linear function of the casein content within the limits of error of the Hill test and the data of Anderson, *et al.* (2) are even more definite on this point. Because of its dilute composition, soft curd milk is lower than hard curd milk in titratable acidity (22), buffer capacity (22, 60, 83),

energy value (26, 60) calcium and phosphorus content (2, 22, 83) and its rate of coagulation with rennin is retarded (40, 71). There appears to be no significant difference between the two types of milk with respect to: pH (22, 60, 71, 83), flocculation point of casein (22, 83), freezing point (22, 83), surface tension (22), and relative viscosity of the whey (83). Whether the proportions of the three different casein fractions found in milk by Svedberg, Carpenter and Carpenter (74) vary as between soft and hard curd milk is not known but has been suggested as a possible difference (83).

The curd tension of milk may be elevated by removing fat in the form of cream or lowered by adding cream (22, 35, 37, 71). Natural high fat milk, being richer in casein as well, usually has a high curd tension compared with natural low fat milk. The curd tension of milk may be lowered by increasing the pH artificially (22, 83) which makes the reaction less suitable for enzyme coagulation.

The opinion of Weisberg *et al.* (83) as to the mechanism in milk which influences and determines the curd character is probably correct and worth reiterating. They hold, that the colloiddally dispersed phase differentiates a soft curd from a hard curd; that concentration and manner of dispersion of the fat, casein, and colloidal phosphates control curd character; that hard curd milk containing more numerous casein particles (and, therefore, coagulation centers) will form a more closely woven net-work on coagulation, resulting in less occlusion of the serum and a denser mass; that fat may contribute to the curd character by interrupting the growth of micellar casein threads, thereby, making a shorter grained texture; and that the degree of dispersion of the fat is as important as its concentration in affecting curd character. This view is compatible with the actions involved in reducing curd tension by various processes and modifications, if the necessity for calcium ions, to render the para caseinate insoluble, is stressed and if the effect of previous coagulation or denaturation of casein and its influence on enzyme action is considered.

*Natural Variations in Curd Tension.*—The curd tension of cows' milk, while shifting somewhat due to stage of lactation, season and other natural causes, is more or less an individual characteristic of the animal and persists from one lactation to another, at least to the extent that composition does not vary (17, 22, 36). Some investigators have not obtained data which would substantiate this statement (5, 66). There seems little doubt, however, that cows carefully selected for the low tension character of their milk, could be assembled and maintained as a herd without excessive turnover of members from one lactation period to another.

The curd tension of milk is high in the colostral period, drops to a low point in the second or third month of lactation, then slowly increases with the duration of lactation, reaching a high level when the milk flow becomes

small, and finally drops to zero when the properties of the milk become abnormal at the extreme end of the period (5, 17, 22, 35, 37, 60, 66). Season and atmospheric conditions affect curd tension in a parallel fashion to composition as would be expected (22, 37, 60). The curd tension is highest in November, December and January and lowest in May, June and July in most sections. Sudden and drastic changes in weather conditions also seem to influence curd character (36). In general it may be said that any natural influence which affects milk composition will be reflected in the curd tension.

**Mastitis:**—Sub-clinical mastitis infections (mild garget) of the udder cause a drop in the curd tension of the milk from the infected udder or quarters (2, 20, 31, 59, 71, 84), the amount varying with the degree of infection (32, 34, 35). This is very evident when milk from clean udder quarters is compared with milk from diseased quarters of the same cow. The decrease in curd tension is frequently sufficient to drop average milk into a soft curd classification (2, 31). Hansen, *et al.* (31) state that an infection caused by staphylococci does not affect curd tension but Anderson *et al.* (2) found that staphylococci infections had even greater effects than streptococci, and in two cases of "coli-like" infection the curd tension was lowered to the greatest degree.

The cause of the decreased curd tension of mastitis milk appears to be due, primarily, to a lowered casein concentration (2, 22, 69, 70), although the higher pH of such milk is also probably a contributing factor (22). Anderson *et al.* (2) have further shown that a lower calcium and phosphorus content and particularly a change in the ratio of casein to calcium to phosphorus in mastitis milk is a significant influence. It should be noted that Dahlberg *et al.* (20) found little change in the curd tension of milk, as a result of sub-clinical mastitis, by the Hill or Miller procedures but noted a decrease when milk was coagulated with pepsin alone. The discrepancy appears to be due to the fact that their samples came from animals very mildly infected as indicated by the normal casein content and the very slight change in other constituents. It is certainly possible to obtain milk from diseased udders which is normal macroscopically and yet will show the definite changes in composition and properties that have been noted by most investigators. In fact Rowland and Zein-El-Dine (69) have proposed a test for mastitis based on the ratio of casein nitrogen to total nitrogen.

In view of the relationship between udder infections and low curd tension values of milk, it is essential in the commercial production and sale of natural soft curd milk to take unusual precautions in selecting uninfected animals when assembling a herd and in keeping the herd free from mastitis during milk production (2, 22, 31).

## MODIFIED AND PROCESSED SOFT CURD MILK

Physicians have practiced milk modification for many years in an effort to find suitable substitutes for mother's milk in the artificial feeding of infants. The literature dealing with these efforts and the results obtained is much too extensive to review here. Let it suffice to say that, as Brenne-mann early pointed out (6), practically all of the successful modifications are effective primarily through their ability to alter the curd character of the coagulum obtained in the stomach; in other words to cause small curd particle size (33). This view has been substantiated in later studies by Lynch (50) and Jeans and Stearns (43), even for acidulated milk.

The boiling of fresh milk for infant use is widely practiced at present and it is the opinion of the Council on Foods of the A. M. A. that "All cow's milk used for the preparation of infant feeding mixtures should be boiled" (18). In addition to boiling or sometimes as a substitute for boiling, such modifications as: dilution; acidification; alkalization; the addition of cereals, lime water and certain colloids; and such processes as homogenization, base-exchange treatment, enzyme alteration or some combination of these, have their advocates.

Only such modifications and processes as can readily be used by the milk dealer in preparing a suitable milk basis for infant formulas will be discussed in this review.

*Dilution.*—When water or milk serum is added to milk the curd tension is decreased (22, 26, 83) more or less in proportion to the degree of dilution (22). Brennemann (6) found that dilution resulted in smaller, more porous curds in the stomach but Wolman (87) states that while dilution reduces curd tension, it does not alter the curd particle size in the "artificial stomach."

*Heat Treatment.*—The effect on curd tension of heating milk depends entirely on the temperature used and the time of exposure. Pasteurization has a negligible effect (5, 11, 22, 76). Heating at 160° F. for 30 minutes causes a distinct lowering of the tension (11), about on a par with a flash treatment at 180° F. (11, 22), while a 30 minute treatment at 180° F. or boiling for 5 minutes decreases the tension markedly, usually sufficient to render average milk (50–60 grams) definitely soft curd (under 30 grams) (11, 22, 76). Autoclaved milk and evaporated milk frequently exhibit no curd tension (22, 37, 61).

The effect of previous heating of milk on its reaction to rennin and pepsin has never been satisfactorily explained due to the obvious complexity of the changes occurring. It seems likely that the calcium ion concentration is decreased, the electrostatic charge carried by the casein micelles increased, some albumin rendered insoluble and the protein itself denatured (67), while at the higher temperatures (autoclaving and sterilizing) an actual heat coagulation of extreme fineness may also be produced (46) which renders the casein more or less immune to enzyme coagulation.

**Acidification.**—As the pH of milk is reduced from the normal (6.4 to 6.7) a toughening of the enzyme curd is observed until the pH drops under approximately 5.9. From this point down to the isoelectric point (pH 4.7) the curd tension decreases (22). This decrease is undoubtedly due to the progressive agglomeration or coagulation of casein in the acid form which interferes with the formation of the para casein curd induced by enzyme action.

It should be noted that the above observations do not apply when the tension is measured by the Hill test (36) using calcium chloride in the coagulant. In the latter case a maximum curd tension is obtained at a considerably lower pH (5.2 to 5.6) (22, 83).

The usual method of acidifying milk for infant use is to add acid (hydrochloric, citric, lactic, acetic, lemon juice, sauerkraut juice, etc.) to such a degree that a fine acid coagulation occurs in cold agitated milk. Little or no enzyme curd will be obtained with such pre-coagulated milk. The use of acid milk has been advocated by many pediatricians, chiefly as a result of the publications of Marriott *et al.* (53, 54, 55, 56). Gonce and Templeton (44) suggest citric acid as being superior for acidifying infant milk.

**Homogenization.**—When whole milk is homogenized the enzyme curd is rendered considerably softer (5, 11, 22, 71, 75, 76, 79, 82, 85). Skim-milk is not so affected (12, 22, 71, 78) which lends support to Weisberg's view concerning the relation of the degree of fat dispersion to curd structure (83).

The effects of homogenization vary with the curd tension of the milk processed, the fat content, the pressure used, and the auxiliary heat treatment. Hard curd milk is softened to a greater degree than soft curd milk although usually not sufficiently to place it in a soft curd classification (5, 11, 22, 75). High fat milk requires higher pressure of homogenization to bring about maximum decreases in curd tension (22, 44). Pressures over 2500–3000 pounds accomplish little (11, 22, 76) with average milk but under this point the tension falls as the pressure is increased, although not proportionately (5, 11, 22, 75, 76). Two stage processing and dual homogenization appear to have little effect not noted with comparable single valve treatment (17, 75, 76).

The temperature treatment of the milk before and during homogenization exercises considerable influence on the results. A greater effect, naturally, is noted when milk is processed at high temperatures due to the additive action of the heat and the homogenization (11, 22, 44, 76) but at very high temperatures (180° F. to boiling) homogenization produces little or no additional lowering of the tension under what is accomplished by the heat alone (44).

Differences between the preheating or pasteurizing temperatures and homogenizing temperatures exercise an influence divorced from the heat

effect itself. Curd tension is reduced to a greater degree when the milk is cooled down to 100°–120° F. before processing rather than to homogenize at the preheating or pasteurizing temperature (11, 22, 85). The greatest reduction appears to be obtained when the temperature range through which the milk is cooled is greatest (22).

All of the above effects have been noted with high pressure, piston machines. At present there are on the market and in use low pressure, rotary homogenizers, which pressure for pressure, seem to have slightly more effect in reducing the curd tension of milk than the piston machines (23). Since their pressures are limited to less than 1000 pounds, they are not capable of producing the amount of change possible with piston machines at 3000 pounds pressure (23, 77).

The curd tension of milk may also be lowered and the product homogenized by conducting it, in a thin layer, over a diaphragm, oscillating at high frequency (12). This process developed by the Submarine Signal Company apparently produces the same general results as are obtained with low pressure homogenization but without any appreciable pressure (12, 13, 23, 28, 87). The method is being used commercially in a few places, the product being known as "sonized" homogenized milk. The amount of experimental work carried on with this process has been rather limited.

Any explanation of the effect of homogenization on the curd tension of milk hinges, without question, on the increased dispersion of the fat which introduces more points of weakness in the coagulum, in line with Weisberg's theory (83). The increase in adsorbed protein may also be a factor since it is quite possible that this membrane protein may not participate in the coagulation in the same manner as it would in the motile state. It has also been noted that the curd from homogenized milk is more highly hydrated than from unhomogenized. This may be a factor in softening the coagulum (29). Again, homogenization may displace some of the normal phospholipide-protein complex from the fat globule surface, releasing it into the plasma in a manner similar to churning. This would affect the tension of the curd (63). Finally, recent work (52) has shown that homogenization raises the freezing point of milk, indicating an adsorption of solutes some of which may be calcium. This would effect the rigidity of the peptic or rennin coagulum under the conditions of the determination.

*Base Exchange Treatment.*—A method was devised a few years ago (48, 49) for retarding enzyme coagulation of milk by the removal of a considerable portion of the soluble calcium. This is a base-exchange process wherein acidified milk (citric acid) is percolated through a zeolite bed, giving up calcium and phosphorus (about 20 per cent) in exchange for sodium and potassium. By properly regulating the conditions, milk with normal pH and normal ratios of calcium to phosphorus and sodium to

potassium can be obtained. Such milk is low in ionized calcium and will usually exhibit a curd tension between zero and 10 grams by the Miller technique. Where the Hill test is used no reduction of curd tension will be evident because of the calcium of the reagent. This product is being marketed in several cities under the name "Sof-Kurd" milk.

One of the frequently voiced objections to "Sof-Kurd" milk is the removal of nutrient minerals, the value of which have been stressed for years and upon which much of the nutritional excellence of milk has been based. However, Hess, Poncher and Woodward (34) in a study using one infant show that the retention of calcium was higher on base-exchange milk than on normal milk. This evidence is hardly sufficient to be conclusive and the subject needs more study.

Another objection to base-exchange milk is the fact that the product is sensitive to temperature changes. Boiling (48) or even heating to temperatures over 100° F., if held for an appreciable time, (29) causes a readjustment of the ionic equilibrium such that curd tension values are considerably increased. The action is reversible and returns to the original value on cooling and holding at low temperature (29, 48). The product is therefore somewhat temperamental and the usual procedures for preparing infant feedings may possibly give rise to variable fluctuations in the curd character of this milk in the stomach.

*Enzyme Treatment.*—Conquest, Turner and Reynolds (16) have proposed a method of reducing the curd tension of milk which employs a period of incubation with an extract of hog pancreas followed by prompt pasteurization. This method is too new to evaluate as yet but seems to offer possibilities. The use of trypsin in similar fashion has been studied by Flora (29) who was able to obtain decreases in curd tension approximately as great as are possible with homogenization, without appreciably affecting the flavor of the milk although, in some cases, the creaming ability was altered.

Treatment of this type is in the nature of a pre-coagulation possibly combined with some denaturation and decomposition of the protein and the effect on a subsequent gastric coagulation is probably not dissimilar from that obtained with acidification, renneting, or high heat treatment.

*Agitation.*—Lundstedt (47) suggested a method of lowering the curd tension of milk by agitation and churning at low temperature. He claimed that the lecithin of the fat globule membrane was removed and adsorbed by the casein thereby modifying the coagulation properties. Palmer and Tarassuk (63) were unable to duplicate Lundstedt's results on agitation but they did show that when the fat globule membrane (phospholipide-protein complex) is removed from the globule in the process of churning, the curd tension of the resultant buttermilk is considerably lowered.

## THE DIGESTIBILITY OF SOFT CURD MILK

The value of soft curd milk, or any modified milk, for infant or invalid use rests on its digestibility characteristics. The curd tension value has been offered as an index of digestibility but has not been definitely proven a satisfactory one. Neither has it been proven definitely unsatisfactory. The size of curd particles formed when milk is coagulated by gastric enzymes is believed to be related to ease of digestion or rate of stomach clearance. Until recently no method of measuring curd particle size was used other than regurgitation of ingested milk and examination of the curds obtained. This index, therefore, also awaits definite substantiation as a measure of milk digestibility. In vitro methods of determining the digestion characteristics of milk have been used to a considerable extent. They are useful in obtaining positive results and in studying phases of the problem difficult or impossible to follow in vivo but procedures have varied so greatly that it is frequently difficult to evaluate the results obtained. In vitro methods which simulate conditions and changes occurring during infant digestion should be productive of useful information but they are always open to the criticism that the conditions are artificial and therefore inconclusive. Methods of rating digestibility utilizing the digestive systems of animals may or may not be more indicative than in vitro methods, depending on the accuracy with which results can be noted or measured and on the similarity existing between the digestive system of the animal and the human infant. In the last analysis any index of digestion must be substantiated clinically with infants; otherwise its meaning and its value are uncertain.

Up to the present only a few studies have produced results which make it possible to accurately evaluate any of the indices discussed and still fewer are available to indicate the usefulness of the more recently suggested methods of processing milk to render it a more suitable base for infant formulas.

*Observations with Humans.*—It is common knowledge that evaporated milk, boiled milk and acidified milk have proven satisfactory as substitutes for breast milk in infant feeding (8, 18, 57, 65). Although all of these milks have very low curd tension values, their success has generally been ascribed to the fineness of the coagulum found in the stomach following their ingestion (8, 43, 50, 64). There seems to be little doubt but that boiled, evaporated or acidified milks are evacuated from the human stomach more rapidly than similar untreated milk (6, 22, 29, 51, 72). This has also been found true with animals as will be indicated later. Ogilvie and Peden, (62) however, found little difference between boiled and raw milk in infants, where stomach tubes were used to sample the gastric contents and Davidson, *et al.* (21) was not able to find appreciable differences between ordinary milk and several types of modified milk (including evapo-

rated) when fed to adults with barium and the rate of stomach emptying noted by means of a fluoroscope. It might be pointed out, however, that Brennemann (6) found in numerous cases that a stomach tube was inadequate for sampling gastric contents and it has never been shown that the admixture of barium sulfate does not greatly alter the characteristics of the milk coagulum in the stomach.

In case studies and clinical observations reported by Hill and others, (4, 17, 36, 37, 39) natural soft curd milk is described as being very satisfactory for infant use, overcoming digestion disturbances such as vomiting, regurgitation, colic and undigested protein in the stools. Elias (25) and Morris and Richardson (60), on the other hand, failed to note any decided advantages for natural soft curd milk over the usual satisfactory formulas. The results reported by these workers are not too indicative inasmuch as Elias' babies ranged up to 2 years in age and the boiled certified milk utilized by Morris and Richardson averaged lower in curd tension than the soft curd milk which was fed raw in most cases. Data presented by the latter workers indicate that their infants on soft curd milk did as well as those on evaporated milk.

Several investigators have noted that natural milk of low tension gives rise to smaller and softer curds in the stomachs of infants and adults (3, 17, 22, 25) as judged by regurgitation tests or the use of stomach pumps. Anthony (3) found the same to be true of homogenized milk but not of base-exchange treated milk.

Homogenized milk and most of the other types of processed milk have been insufficiently studied with humans to make conclusions possible. Wolman (87) states, apparently as a result of *in vitro* studies, that adequately homogenized milk is an excellent foundation for infant formulas and Wilcox (86) reports, as a result of Roentgenological studies with adults, that homogenized milk leaves the stomach in advance of soft curd milk and average milk.

The common conception that the fat of homogenized milk is more assimilable by infants because of its greater dispersion seems unfounded in view of the studies of Holt and co-workers (41).

Rogers, *et al.* (68) found base-exchange treated milk to be a good complementary food for new born infants, better gains and fewer losses resulting than where untreated milk was used and, as previously noted, one very limited study (34) indicated that the lack of calcium and phosphorus in base-exchange treated milk does not impair its nutritive value since the remaining minerals are assimilated to a greater degree.

*Observations with Animals.*—Digestion comparisons have been made with soft curd milk, boiled milk, acidified milk, evaporated milk and a few of the other types of modified milk using such animals as dogs, calves and rats. Some of the results obtained appear quite indicative but there

have been far too few such studies to warrant placing much weight on them, particularly since the digestive apparatus of calves is quite dissimilar from humans and the stomachs of adult dogs appear not to coagulate milk normally (33).

Working with dogs, Espe and Dye (26) concluded that soft curd milk leaves the stomach quicker than normal or hard curd milk. The same conclusion has been reached in other studies using calves (22, 27, 61) and rats (22) and for boiled milk and whole milk as compared with unboiled milk and skim milk respectively (27, 61). It has also been shown that soft curd milk, buttermilk and evaporated milk travel farther and disappear more rapidly in the intestines of rats than does hard curd milk (22). Hess and co-workers (33) found that modified milks (boiled and acidified) form loose, small curds in the stomachs of puppies, whereas raw and pasteurized milks form large tough coagula. Milk treated with enzymes of the hog pancreas to reduce the curd tension and fed to calves was observed by Conquest, *et al.* (16) to have an increased rate of stomach clearance compared with normal untreated milk.

In some recent studies, Flora (29) observed that natural untreated milk is digested by rats at a rate roughly proportional to the curd tension but that homogenized milk (by whatever method processed) and to a much lesser degree, base-exchange treated milk digest at a slower rate than the respective curd tensions would seem to indicate.

*In Vitro Studies.*—It is difficult to decide how much confidence to place in the methods which have been devised for measuring the digestibility of milk by laboratory means inasmuch as clinical substantiation of the results is usually lacking and inasmuch as little information has been obtained regarding the actual conditions under which milk coagulates in the infant stomach. Marriott and Davidson (54) present the following data regarding the pH of the infant stomach at the height of digestion (2 hours after feeding).

	<i>Breast Fed</i>	<i>Cows Milk</i>	<i>Acid Milk</i>
Normal ... ..	3.75	5.10	3.71
Abnormal ... ..	4.74	5.35	4.10

These values are much higher than obtain in adult digestion (32) and since it has been shown that cows milk is coagulated in adults at a pH of 5.9 or higher 10 minutes after ingesting one pint of milk on an empty stomach, (22) it seems reasonable to believe that coagulation in the infant stomach takes place at a pH at least this high and perhaps higher.

A "rennin type" curd is obtained when milk is coagulated with peptic enzymes at a pH of 6.0 or above. Between pH 5.0 and pH 6.0 the coagulum appears to be a mixture of the acid and rennin types, while at a pH of less than 5.0 the curd is predominately acid in type. The character of the coagulum (curd tension and curd particle size) will vary with the

reaction at which it forms (22, 42) as will also its digestion properties (42). It therefore appears evident that, in methods for following rates of digestion in vitro, the conditions under which the milk is coagulated are of prime importance and should simulate the conditions in the infant stomach as closely as possible. Furthermore it is the writers belief that the conditions should conform to those found in the new born rather than to those existing in babies six months or over in age. It seems axiomatic that any type of milk which proves satisfactory in new born babies will cause no difficulties with older ones. It is in the very young that most difficulties with cows' milk are experienced.

Hess *et al.* (33), using an in vitro method set up to simulate stomach conditions concluded that, while curd particle size is a most important factor in digestion, other influences are also of moment. They found that boiling milk lowers the soluble nitrogen content as does also acidification, but both of these factors favor peptic digestion. This in itself is evidence that the physical character of the curd is of first importance and overshadows other lesser effects. Schultz and Fetter (73) found that milk containing rennin (Junket) is acted upon more rapidly by pepsin. Wallen-Lawrence and Koch (80) observed that heated milks (boiled and evaporated) are attacked more rapidly by trypsin than unheated milk and explained this as being the result of the destruction (by heat) of a labile trypsin inhibitor found in the whey of raw milk. Doan and Welch (22) showed that soft curd milk digests faster at any pH than does hard curd milk and that peptic digestion appears to be a peripheral process as far as nitrogen break down is concerned. Lear and Skaggs (45) found that natural soft curd milk has superior digestion qualities to normal milk and that boiled milk and homogenized milk have inferior qualities. Their results with boiled milk were due to the fact that soluble nitrogen was taken as an index of stomach digestibility and, as has been indicated (33), boiling itself lowers the soluble nitrogen. Chambers and Wolman (14) and Wolman (87) have reported enhanced digestibility for homogenized milk, heated milk, natural soft curd milk and various types of modified milk. Their method made use of thin walled rubber bags ("artificial stomachs"), in which a type of agitation more nearly approaching peristalsis was obtained. Their measure of digestibility was the curd particle size or the curd surface area which resulted when the various milk samples were treated under uniform conditions to simulate coagulation and the first stages of digestion in the stomach. Their results indicate that curd tension correlates quite satisfactorily with curd particle size except with a few unusual types of modified milk. A pH of 4.5 which was used in these studies might be criticized as being too low, particularly when used as the coagulation reaction.

Studies recently reported by Hull (42) show that homogenized milk does not digest any more easily than unhomogenized milk but that evaporated milk, base-exchange treated milk and boiled milk do have enhanced digestion qualities. His results indicate that the pH at which the curd is formed has a very definite influence on the curd particle size and hence on digestibility. At the lower pH levels (5.2–5.7) the curd is less adhesive and in smaller aggregates and the breakdown is more rapid than at the higher levels (5.9–6.4). Flora's (29) work substantiates that of Hull, in many respects, although in the *in vitro* method used, tryptic digestion followed peptic and the results obtained were checked utilizing rats. He concludes that homogenized milk digests no better than unhomogenized, that curd tension is not an accurate index of digestibility with this type of milk or with base-exchange treated milk, that enzyme treated milk shows good peptic digestion characteristics and that any milk to be highly satisfactory as a base for infant formulas should have a curd tension of zero. In addition Flora noted increased tryptic activity with the heated milks (boiled and evaporated) in line with the findings of Wallen-Lawrence and Koch (80).

The available information on the digestibility of natural and processed or modified soft curd milk is inadequate to be conclusive. In some respects it is conflicting. There seems little doubt but that natural soft curd milk is more suitable for infant feeding than normal or hard curd milk but whether it is sufficiently more digestible to warrant its production is questionable since it does not seem to offer any definite advantages over evaporated milk, acidified milk and perhaps most boiled milk.

Homogenized milk (including sonized) has not reacted very favorably in some *in vitro* studies so that until further studies are made and particularly until careful clinical comparisons are available little can be offered in support of it. Base-exchange treated milk also needs further substantiation as an entirely satisfactory infant food. Present information would indicate that it is probably in a class with natural soft curd milk in that it seems to be more digestible than normal milk but not so digestible as evaporated and acidified milk.

Enzyme treated milk has been studied even less than the others. Results obtained by one or two workers indicate that it may have possibilities in infant feeding if and when a satisfactory method of preparing it is developed.

Much doubt has been cast on the curd tension value of milk as a satisfactory index of digestibility. It is the writer's opinion that the *size* of curd particles obtained in peptic coagulation under conditions of agitation and acidity closely approximating those existing in the stomachs of young infants would be a more suitable index than the *toughness* of the curd formed without any agitation. However, the pH at which coagulation is

to be accomplished would be a factor needing substantiation and one to be accurately controlled. Observations on this point with infants are lacking.

In conclusion it should be emphasized that, at present, none of the suggested methods of preparing a fresh fluid milk for pediatric purposes appears to be sufficiently satisfactory. At least evidence in their favor is too meagre or too fallible to constitute definite proof of their sufficiency. Instead of being content to promote the use of a half-satisfactory product or a product only half substantiated, the milk industry should redouble its efforts to develop a type of fresh cow's milk which will meet every requirement of the human infant or to thoroughly prove the excellence of existing types in a manner acceptable to pediatricians.

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# American Dairy Science Association Announcements

## RESULTS OF ELECTION

The results of the election of officers on October 1 were as follows:

Vice President: E. S. Guthrie, Cornell University, Ithaca, N. Y.

Directors to serve for three years each:

J. W. Linn, College of Agriculture, Manhattan, Kansas

M. E. Parker, Beatrice Creamery Co.,  
1526 South State Street, Chicago Illinois

## ANNUAL MEETING AT IDAHO AND WASHINGTON, WEEK OF JUNE 26, 1939

Our next annual meeting is to be held the week of June 26, in Moscow, Idaho, and Pullman, Washington. Will you please inform the secretary if you are interested to go by train and if so, would you use Pullman or Tourist Pullman beyond Chicago? A card stating your preference will in no way obligate you for the trip. This is merely to find out if it is worth turning over to the railroads who will close any contract if made. If enough interest is shown, we may arrange to have a special car or train so that you may go from the East to the meeting together.

	New York	Washing- ton	Colum- bus	Chicago
Rail Fares Using Pullman	\$128.40	\$120.50	\$94.20	\$76.05
“ “ “ Tourist Pullman beyond Chicago	118.45	110.55	74.25	66.10
Pullman Fares	20.00	19.50	16.50	14.00
“ “ Using Tourist Pullman beyond Chicago	13.50	13.00	10.00	7.50

## A REVIEW ARTICLE

Beginning with the October issue, the editor has invited authorities to write on a specific subject. From six to twelve of these review articles will appear each year. In the October issue, Mr. C. J. Babcock has covered the subject of feed flavors in milk and milk products. We believe these review articles will be a great asset to the JOURNAL.

## ASSOCIATION NEWS

Upon receipt of your JOURNAL will you please look for Association announcements? They will be found immediately preceding the abstracts.

Your officers will appreciate suggestions and criticisms in operating the Association, publishing the JOURNAL, etc.

We should appreciate your comment regarding the exhibit displayed at the National Dairy Show and the Dairy Industries Exposition.

**BORDEN AWARD**

Have you anyone in mind to be nominated for the Borden Award? These committees will no doubt be asking for nominations in the immediate future.

# JOURNAL OF DAIRY SCIENCE

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## SULFANILAMIDE IN THE TREATMENT OF STREPTOCOCCIC MASTITIS\*

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### INTRODUCTION

Much interest is being shown at the present time in the use of sulfanilamide in the treatment of streptococcic mastitis of cattle. In the published reports to date, variable results have been obtained by the use of this drug. These facts indicated that further study of this problem should be made.

### REVIEW OF LITERATURE

Domagk (4) in 1935 first reported the favorable effect of certain Azo dyes in mice experimentally infected with streptococci. The name "sulfanilamide" was later given to the essential nucleus or effective portion of these dyes by the American Medical Association. Numerous results on the use of sulfanilamide in human medicine have been reported.

Very few controlled experiments with the use of this drug in streptococcic mastitis of cattle have been reported. Allot (1) in November, 1937, reported both clinical and laboratory evidence of improvement in three cows treated with approximately the dose recommended for man, that is, 1 gram for each 20 pounds of body weight. In each instance the case relapsed to approximately its previous condition after treatment was discontinued. Several case reports found in Cattle Breed Journals and Biological Journals have reported favorable results from the use of this drug in doses of 15 to 45 grains (15 grains = 1 gram) 2 to 4 times daily for 3 to 7 days.

Baer and Gunderson (2), reporting on the use of sulfanilamide in the treatment of mastitis, state that streptococci did not appear during treatment but were present after treatment was discontinued on two cows and that streptococci were reduced following treatment on two other cows.

Scholz (8) reported on four cases of streptococcic mastitis treated with sulfanilamide at the California Veterinary Conference, January, 1938.

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These represented slight, moderate, severe and very severe cases of mastitis. The cows averaged approximately 900 pounds each and were first treated with 120 to 150 grains (8 to 10 grams) of sulfanilamide in two equally divided doses daily. The only case that showed any improvement was the one showing slight evidence of mastitis. After a 7-day rest period, the four cases were again treated for 7 days with sulfanilamide in 2 equally divided doses, with the mild case receiving 80 grams daily and the other 3 cases 40 grams daily. The streptococci disappeared from the milk of all quarters in the mild case and from 3 quarters of the moderate case and were reduced in number in the severe and very severe cases. Two weeks after treatment streptococci were again present in all quarters of all the cows treated. No symptoms of intoxication were observed in any of the cows.

Schlotthauer (9) reported on the treatment of one cow for streptococcic mastitis with sulfanilamide at the Intermountain Livestock Sanitary Association meeting in January, 1938. He stated that one 900-pound cow was treated for 3 days with 90 grains (6 grams) daily and showed 1 mg. of the drug in 100 cc. of the milk. She was then treated with 44 grams daily for 4 days. In the neighborhood of 8 hours after the last dose, she showed 5.6 mg. per 100 cc. of the milk. The highest per cent of the drug was found in the normal quarter and much less, or about 1 mg. per 100 cc., in the inflamed quarter. In both treatments there was inhibition of the organisms in the udder but they were not destroyed.

Recently Johnson and Miller (5) have reported on the use of 75 grains (5 grams) of sulfanilamide twice daily for 3 days on 6 cows and  $\frac{1}{2}$  ounce (14 grams) twice daily for 8 days on 4 cows afflicted with chronic streptococcic mastitis with no apparent beneficial effects from the treatment. No toxic effects were produced by the treatment.

#### PURPOSE OF INVESTIGATION

The study at the Idaho station was started in June, 1937, before the above reports were presented, and because of the absence of accurate data then available it seemed advisable to study the following points:

1. To determine how often it would be necessary to administer sulfanilamide to cattle to maintain a reasonably constant level in the blood.
2. To find what dose is necessary to attain a blood concentration in cattle comparable with that obtained in man.
3. To establish the degree of effectiveness of the treatment in streptococcic mastitis, and
4. To determine what unfavorable results, if any, follow its use.

Streptococcic mastitis has been studied in this station herd for the past ten years. During the past fall and winter, a particularly virulent form of the disease has been prevalent. Heifers showing their first clinical symptoms drop in milk production and go off feed. Difficulty is experienced in getting the affected quarters back to normal with past methods of treatment.

## DOSE INTERVAL TO MAINTAIN BLOOD LEVEL

In this work, "Stramid," a brand of sulfanilamide marketed by the Alba Pharmaceutical Company, was used. In every instance the material was given by mouth in gelatin capsules. The concentration of unconjugated sulfanilamide in the blood and urine was determined by the method recommended by Marshall, Emerson and Cutting (6). The Marshall, Emerson and Cutting method was adapted to the determination of the concentration of unconjugated sulfanilamide in the milk.

Two cows were used in this phase of the study. A Jersey cow, #107X, was given 5 grams of sulfanilamide for each 100 pounds of body weight. Blood, urine and milk samples were taken at hourly intervals following this dosage and the sulfanilamide concentration determined. Similar data were also taken on a Holstein cow #55X following the last dosage of a 7-day period of treatment. In both instances it was found that sulfanilamide

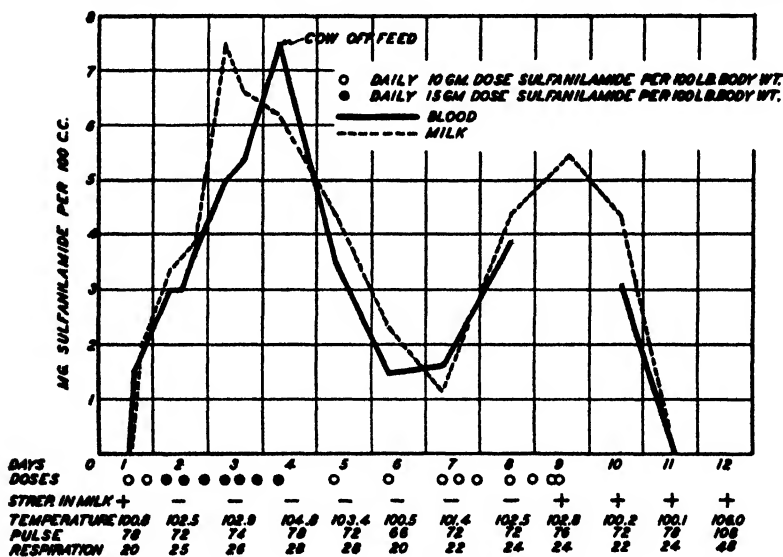


FIGURE 1. Cow #8X. This graph shows the variations in doses given, the fluctuations in the blood and milk levels of sulfanilamide. The presence or absence of hemolytic streptococci in the milk and the effect of the treatment on the temperature, pulse and respiration are also shown. The cow showed symptoms of sulfanilamide poisoning when the blood concentration was between 7 and 8 mg. per 100 cc. This cow died showing cyanosis of the voluntary muscles the day following the last shown in the table.

concentration in the blood did not start to drop until about 12 hours after the last dose was administered. Seldom was sulfanilamide detected in the blood or milk 48 hours after the last dosage (See Figures 1 and 2).

The greater persistence of blood level of sulfanilamide in cattle than in man is not surprising, since in ruminants the transfer of material from the rumen is slow, allowing the drug to be presented slowly for absorption

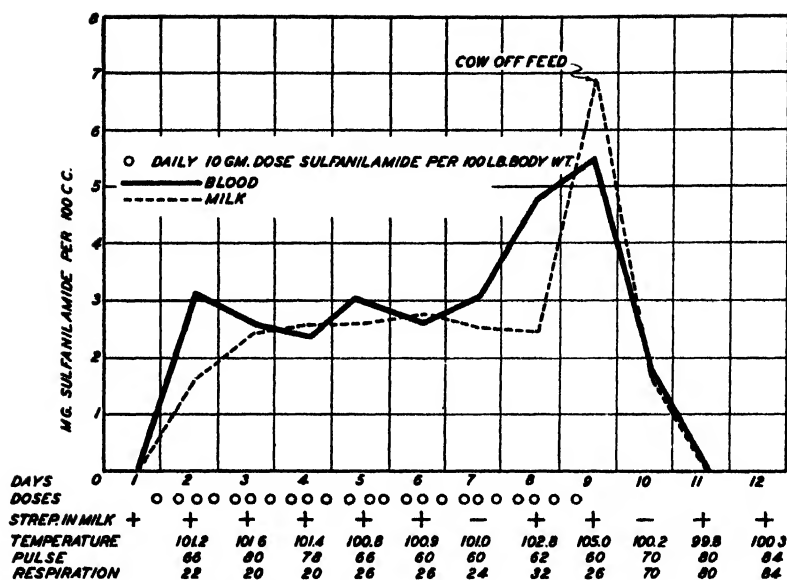


FIGURE 2. Cow #89X. This shows the general level of sulfanilamide in the blood and milk when a cow receives 10 grams per 100 pounds body weight daily. On the last day of treatment the cow showed symptoms of intoxication. At this time the blood level was about 5.5 mg. and the milk level just under 7 mg. per 100 cc.

to the small intestines. This study would indicate that it was not necessary to repeat the dose of sulfanilamide more often than every 12 hours or twice daily.

#### BLOOD LEVEL IN RELATION TO DOSE

In the initial trial, where the dosage was at the rate of 5 grams of sulfanilamide to 100 pounds of body weight daily, the blood and milk level remained slightly below 2 mg. per 100 cc. The drug was administered in three or four equally divided doses. This level was far below the 10 to 20 mg. attained in man (6). On subsequent trials, the dose was doubled and trebled that used in man; that is, 10 and 15 grams per 100 pounds body weight. Reference to Figures 1 and 2 show that with these high doses, it was possible to attain blood levels slightly below 8 mg. per 100 cc. Cows regularly go off feed and show other clinical symptoms of sulfanilamide poisoning before the blood level reaches 8 mg. per 100 cc. From this study it would seem impossible to attain blood levels in cattle comparable with that attained in man. This may be due in part to the rapid excretion of this material in the urine or to the possibility of a greater conjugation of sulfanilamide in the blood of cattle.

#### EFFECTIVENESS OF SULFANILAMIDE IN TREATING STREPTOCOCCIC MASTITIS

Three distinct stages of streptococcic mastitis cases were treated with varying levels of sulfanilamide. Two Jersey cows (#107X and #112X)

had streptococcic infection of about 8 months' duration, one of which showed definite clinical symptoms, were treated with 10 grams per 100 pounds body weight for 7 to 10 days. Streptococci were absent from the milk during most of the period of treatment, but were detected following the termination of treatment. No. 107X had previously been treated with 5 grams per 100 pounds for 9 days without permanently eliminating the streptococci.

Failure to eliminate the causal organisms in these two cases prompted the treatment of two Holstein cows (#89X and #74X). These cows had contracted streptococcic infection of the udder during the previous 2 months as shown by bacteriological examination of the milk. No clinical evidence of mastitis had developed in either of these cows. They were treated for 8 days with 10 grams per 100 pounds body weight. Streptococci were absent from the milk of #74X during treatment but were present in the milk of #89X. Streptococci were present in the milk of both cows continuously after the termination of treatment. Apparently sulfanilamide even in doses twice that recommended for humans is not effective in eliminating streptococci from the udder of cows affected with non-clinical streptococcic mastitis.

Several cases of acute clinical streptococcic mastitis (#24X, #8X, #85X, #194, #101X, #89X, #128X, #126X, #87X, #103X, #175, and #187) some of which were flare-ups of old chronic cases and others of recent infection were treated with 5 to 10 grams of sulfanilamide per 100 pounds of body weight for periods of 7 to 10 days. Most of these cases had been treated with hot packs, frequent milking, udder ointments, massage, and mild laxatives without eliminating the tendency for the milk to become watery or thick with pus. In some instances the fever, swelling and hardness in the affected quarters were practically eliminated by this standard treatment.

By the end of the sulfanilamide treatment, all cases except one were showing improvement in the consistency of the milk, although many of them were deficient in quantity of milk from the affected quarters.

Treatment with sulfanilamide was successful in restoring normal flow and normal appearance in the milk and the quarter if administered in the early stages when the first symptoms of flaky milk and congestion of the quarter appear. It did not eliminate the streptococci from the udder, nor prevent later acute attacks. This treatment, however, apparently saved many quarters which otherwise would have been lost by reducing the acute infection before the quarter was permanently injured.

Treatment was also effective in restoring normal appearance to the milk of recently infected cows when the condition had developed to the stage where the milk was discolored and watery. However, in these severe cases the affected quarters shrunk to half the normal size and the milk flow was reduced about 50 per cent. One exception to the above (#103X)

showed only partial recovery and relapsed to the previous state after treatment was discontinued.

Use of sulfanilamide resulted in improvement in acute attacks of old chronic cases, but normal milk was not secured in all instances. In one case out of three the milk failed to return to normal appearance.

Five grams of sulfanilamide per 100 pounds of body weight seemed just as effective in producing favorable results as did larger doses.

#### TOXICITY OF SULFANILAMIDE

Sulfanilamide in doses of 5 grams per 100 pounds body weight, when administered in three equally divided and spaced doses each day over a period up to 12 days, had little or no detrimental effect in 9 of 11 cases treated. This amount did not reduce milk production.

Twice this amount, that is, 10 grams per 100 pounds body weight, after 5 to 7 days' administration, is sufficient to cause distinct toxic effects in the form of general sluggishness, reduction in feed consumption, reduction in milk flow, rough coat and occasionally complete loss of appetite and increased temperature and respiration. (See Figures 1 and 2.)

Higher amounts, that is, 15 grams per 100 pounds body weight daily, for from 1 to 3 days, produce the above-mentioned toxic symptoms accompanied by a precipitate of crystals in the urine. Reduction in milk flow associated with toxic doses was commonly observed. When treatment is discontinued, the milk flow rapidly returns to normal.

One cow (#8X) died the third day following discontinuance of treatment with 10 and 15 grams sulfanilamide per 100 pounds body weight. She became foundered on not more than 10 pounds of grain, developed a diarrhea and died within 36 hours. On post mortem, the liver, spleen and kidneys appeared normal. Definite evidence of acute enteritis was present. All muscular organs were extremely cyanotic, particularly the voluntary muscles which were quite blue on dissection, but soon became normal flesh-colored when exposed to the air. This condition has been found in man associated with sulfanilamide poisoning as recorded by Bigler, Clifton and Werner (3).

One cow (#112X) which was turned out during the last three days of a 10-day period of treatment with 10 grams per 100 pounds body weight, developed an eczema with considerable loose scab production over the head, neck and shoulder and between the limbs and on the udder. The condition seemed to be most severe where there was contact with the stanchion. A similar condition is common in man as reported by Newman and Sharlit (7) when individuals being treated with sulfanilamide are exposed to the direct rays of the sun.

One cow (#126X) died of bloat while on treatment with 5 grams per 100 pounds body weight. She had shown no symptoms of sulfanilamide poison-

ing and did not show any post mortem evidence of poisoning. She is believed to have died of simple bloat.

#### SUMMARY

1. The blood and milk levels of unconjugated sulfanilamide in cattle were maintained over a period of 12 hours either following an initial dose or after the last dose of a period of treatment. This insures a reasonably constant level of sulfanilamide in the blood of cows that are dosed twice daily at 12-hour intervals.

2. It was possible to attain a level of sulfanilamide in blood and milk slightly under 8 mg. per 100 cc. only when the dose was approximately 10 grams per 100 pounds body weight or twice that recommended for man. Blood levels slightly less than 2 mg. per 100 cc. were attained with a dose comparable with that recommended for man, that is, 5 grams per 100 pounds body weight daily. Both of these levels are below that of 10 mg. per 100 cc. of blood suggested for favorable results in man.

3. Doses of 5, 10, or even 15 gm. per 100 pounds body weight over a period of 3 to 10 days failed to permanently eliminate Beta Hemolytic Streptococci from the udders of cows affected with streptococcic mastitis, regardless of whether the cases were acute or chronic or of short or long duration. Even recently affected non-clinical cases were not freed of the organism.

4. Symptoms of acute streptococcic mastitis such as tenderness, swelling, hardness of the quarter, accompanied by flaky, pussy, or watery milk were relieved in most cases by administering sulfanilamide in doses of 5 to 10 grams per 100 pounds body weight for 7 to 10 days. Five grams per 100 pounds body weight seemed to be as effective as larger doses in relieving clinical symptoms of acute mastitis. The cases treated had failed to respond satisfactorily to the standard treatment of applying hot packs, frequent milkings, massages, laxatives and udder ointments.

5. Sulfanilamide poisoning in the form of sluggishness, loss of appetite, reduced milk flow, roughened coat, fever and increased pulse and respiration were produced in 1 to 3 days when the total daily dose was 15 grams per 100 pounds body weight. Five grams per 100 pounds body weight had little or no detrimental effect in 9 of 11 cows treated. The dose should be reduced or eliminated when toxic symptoms appear.

6. One cow died following doses of 10 and 15 grams per 100 pounds body weight with enteritis diarrhea and definite cyanosis of the musculature. Another cow showed extensive eczema when allowed contact with the direct rays of the sun during treatment with 10, and later 5, grams per 100 pounds body weight. There seems to be an individual difference in tolerance of cows to sulfanilamide.

7. Sixteen cows were treated for streptococcic mastitis with sulfanilamide. Eight of nine cases with an initial infection showing acute symptoms gave favorable results with a reduction of inflammation of the udder and restoration of normal-appearing milk. One cow showed symptoms of toxic poisoning and treatment was discontinued. Favorable results were obtained in 6 out of 9 severely affected quarters in 4 old chronic cases. The 3 additional quarters were greatly improved. No improvement was shown in three cases of initial infection where clinical symptoms had not developed.

The bacteriological and chemical phases of this study were conducted with the cooperation of Walter G. Hoge, W. V. Halversen, and V. A. Cherrington of the Department of Bacteriology, University of Idaho. A report covering this phase of the study in more detail will be published as Research Paper No. 169.

*Note:* Individual case histories of cows treated in this study are available to anyone upon request to the authors.

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# AN X-RAY DIFFRACTION ANALYSIS OF CASEIN

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AND

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## HISTORICAL INTRODUCTION

### *X-Ray Studies of Protein Structure*

The purpose of x-ray analysis is to determine the arrangement of diffracting units within a crystal unit and to interpret the properties of that crystal in terms of that arrangement. Proteins, in general, are not crystalline in structure, according to Astbury and Lomax (1), and, consequently, typical crystalline effects are not registered on a photographic film when they are subjected to x-ray analysis. Meyer and Mark (2) were the first to apply the principles deduced from the structural arrangement of cellulose to their interpretation of the protein pattern of fibroin. At that time they suggested that fibroin was made up of extended polypeptide chains, built for the most part of alternating glycine and alanine residues. This type of structure has been confirmed for fibered proteins by Astbury (3) who has studied the protein keratin from a number of different sources and under a wide range of conditions. Astbury found that hair keratin existed in two structural forms. The pattern for stretched hair, or beta keratin, could be explained in terms of extended polypeptide chains, whereas the pattern for the unstretched hair or alpha keratin was one of random arrangement. Therefore, this indicated that the chains were in a crumpled state.

Non-fibrous proteins give patterns that cannot be used as successfully for unit cell structure determination as can patterns obtained from fibered proteins. Casein is not a fibered protein, according to Svedberg, Carpenter, and Carpenter (4), but exists as small particles almost spherical in shape. The particle size was determined by Svedberg (4) by calculation from specific sedimentation velocity data using Stoke's law, under the assumption that the particle was spherical. The value for radius is  $(r) = 4.177 \times 10^{-7}$  cm. (41.77 Å). Using the diffusion constant and Einstein's law, particle size was also determined when no assumption was made as to the shape of the particle. The value for  $r$  was then found to be  $r = 5.994 \times 10^{-7}$  cm. (59.94 Å). The ratio between the two values of  $r_E/r_S$  equals 1.43 and this figure agrees well with the values for hemoglobin, serum albumin, and serum

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\* The data presented in this paper are from a thesis submitted to the Graduate School of the University of Illinois in partial fulfillment of the degree of Doctor of Philosophy, 1937.

globulin that had been determined previously by Svedberg and are known to be non-fibrous.

At times difficulty in interpreting the physical properties of casein and compounds of casein has been encountered. Recently x-ray analysis has been applied to research in the biological fields where valuable contributions have been made by this technique to the knowledge of the structure of certain proteins. In undertaking this study it was considered that x-ray analysis would yield information that could be secured in no other way. If changes in structure of the casein molecule could be correlated with definite treatments, then possibly the properties of this compound could be accounted for by the molecular arrangement.

#### EXPERIMENTAL PROCEDURE

##### *Methods for Casein Preparation*

Pure casein was prepared according to the method of Van Slyke and Baker (5). Although Svedberg, Carpenter, and Carpenter (6) have shown that casein prepared by the Van Slyke and Baker method does not correspond in molecular weight with casein prepared by Hammersten's procedure, nevertheless their results show that casein prepared by either method is a mixture of protein molecules of varying molecular weights. The main constituent of Van Slyke's and Baker's casein, according to Svedberg (6), corresponds to a molecular weight of 100,300, whereas approximately one-third of the casein prepared by Hammersten's method has a molecular weight of 375,000.

Casein was also prepared by hydrochloric acid precipitation from fat-free milk by adjustment of the pH to 4.6. Casein products prepared by rennin coagulation, ultra-filtration, ultra-centrifuging, and electric deposition were also used.

Various addition compounds of casein were prepared from the Van Slyke-Baker casein. The following chemicals were used for their preparation: NaOH, Na<sub>2</sub>CO<sub>3</sub>, NH<sub>4</sub>OH, Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>, and 40 per cent formaldehyde. The procedures followed were those published by Sutermeister (7).

##### *X-Ray Analysis*

For x-ray analysis the usual diffraction technique was used. X-rays were generated in a Philips metallix type tube, using a copper target. The majority of exposures were taken when the tube was being operated at 35 KV and 25 Ma. X-rays from an iron target were also tried. The tube was then operated at 18 KV and 15 Ma. The powder method of Hull, Debye, and Scherrer was used with the sample mounted directly in front of the .025 inch lead pin hole. The exposures lasted from 3 to 6 hours. Each pattern was registered on a flat film held in a cassette 5 cm. from the sample. Figure 1 illustrates, by a diagrammatic drawing, the laboratory technique that was

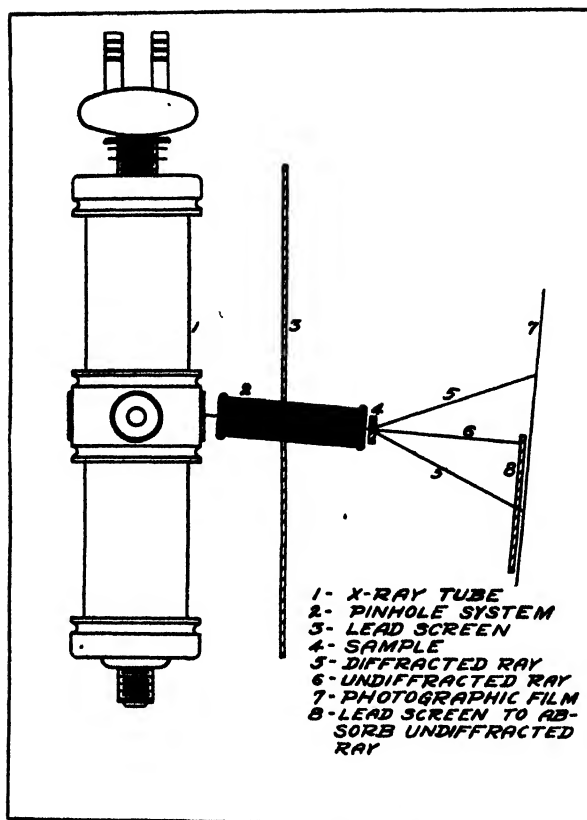


FIG. 1. Diagrammatic sketch of x-ray tube.

used for the x-ray diffraction analysis of the substances studied. The same diffraction pattern was obtained regardless of whether a thin sample (.1 to 1 mm.) of dried casein was used or whether the sample was first ground to a powder with mortar and pestle. When the materials were not dried broad diffuse halos were obtained instead of the characteristic pattern of the dried casein samples.

Interpretations of the powder patterns obtained from the various substances were made by the use of the Bragg equation,  $n\lambda = 2d \sin \theta$ . The radii of the diffraction rings were determined and then by appropriate calculations and substitution in the above equation the "d" values, or distances between diffraction units or planes, were calculated. The "d" values are specific and characteristic for each substance. This technique, therefore, serves as a ready means for analysis and identification of materials.

#### EXPERIMENTAL RESULTS AND DISCUSSION

##### *X-Ray Diffraction Analysis of Casein*

Pure casein, made by the Van Slyke and Baker (5) method, was the first

to be subjected to x-ray analysis by the pin-hole method. Following this, caseins made by the procedures previously described were analyzed. The diffraction patterns obtained from each of these products had two halos characteristic of complex proteins whose component groups are apparently not arranged in a geometric fashion, but whose arrangements are entirely ones of random. Although Figures 2 and 3 illustrate only the patterns ob-

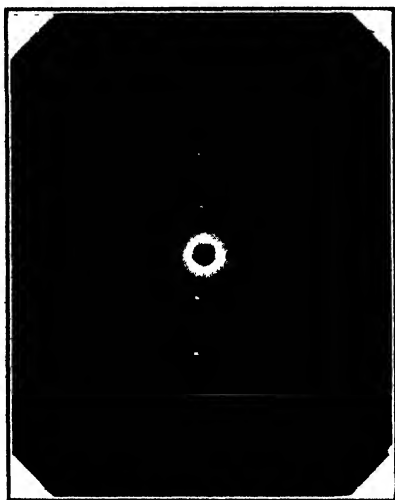


FIG. 2. Pure casein.

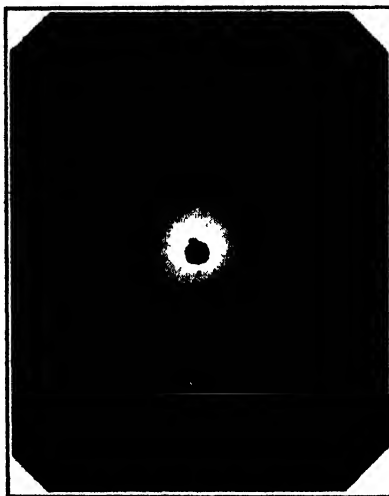


FIG. 3. Casein coagulated by rennin.

tained from pure casein and casein coagulated by rennin, the patterns from the other caseins are almost identical. The calculated spacings for  $d_1$  and  $d_2$  of these caseins are of the same order of magnitude, which is evidence of their similarity. The values are listed in the following table.

TABLE 1  
*Spacing values for caseins*

Spacing	Pure casein Van Slyke and Baker	Crude com. casein ppt. by HCl	Casein coagulated by rennin	Casein from ultra filter	Casein from ultra centrifuge	Casein deposited by elect. current
$d_1$ .....	9.9 Å	9.8 Å	9.9 Å	9.9 Å	9.84 Å	9.7 Å
$d_2$ .....	4.66 Å	4.60 Å	4.62 Å	4.60 Å	4.65 Å	4.60 Å

Although the above products were prepared by entirely different procedures the method of preparation had no marked influence on the internal arrangement and molecular structure of the caseins, as determined by x-ray diffraction analysis.

The diffraction pattern obtained from casein (1, 8) is typical of non-fibrous proteins which are without sufficient orientation or number of inter-

ferences to lend themselves readily to accurate analysis, and as a result no satisfactory solution of structure has been developed. The probable explanation of the lack of structural organization in casein and other proteins of a like nature, according to Clark and Schaad (9), is due to the mutual attraction of the large number of polar groups that probably occur in a complex protein structure. The polar groups are apparently distributed at irregular intervals in both side chains and along main chains; hence the structural design is not one of a regular geometric pattern but is one due to the chemical nature of the material.

Emil Fischer was the first to advance the hypothesis that proteins are essentially a synthesis of polypeptide chains, and since the work of Meyer and Mark (2) x-ray diffraction data of protein structure has been interpreted on the assumption that proteins are built up of polypeptide chains which are either in an extended condition, as in stretched hair, or in a crumpled state, as in unstretched hair. X-ray data, as interpreted by Astbury (10), have also shown that side chains extend from the main chain at regular intervals and these serve as definite diffraction units in determining the perpendicular distance, in the same plane, between the polypeptide chains. This spacing is exceedingly varied since it seems to depend to a large extent on the amount of water held by the protein. For the majority of proteins this value has been calculated to be from 9.5 Å to 10.5 Å, although Clark and Schaad (9) have found it to vary from 10.4 Å to 17 Å for certain proteins, depending on the amount of water bound by the protein. On the other hand, it can be seen from Table 1 that this spacing was very uniform in the different casein samples analyzed.

In addition to this 9.8 Å spacing which is always present in diffraction patterns of proteins, another one appears which represents the perpendicular distance or thickness between planes of polypeptide chains. This value is rather uniform for all proteins, being for the majority 4.6 Å. The casein samples showed no exception for this spacing, and all samples were very uniform in this spacing value. Secondary valence forces are assumed to be responsible for the holding together of the polypeptide chains in the protein grid (10, 11, 12).

In the production of the peptide linkage there is a combination of the amino group of one amino acid with the carboxyl group of another, to yield the CONH group. Apparently the alternation of CONH and RCH groups determines the repeating patterns of proteins. The characteristic identity period for silk, hair, and other proteins has been of the magnitude of 3.5 Å (10). A spacing at 3.08 Å has been found to be present in cheese but not in casein.

Although the peptide linkage seems to be the most common and prominent type of binding proteins, Abderhalden's diketopiperazine hypothesis

has been used to explain a few peculiar reactions of proteins to certain treatments and reagents.

### *The Reaction of Casein to Certain Alkaline Reagents*

In order to determine the effect of alkalis on casein, alkaline caseinate compounds were prepared as previously described. Each of these gave the same x-ray diffraction pattern and, upon calculation, the "d" spacings were approximately the same as for uncombined casein. This would indicate, then, that the preparation of sodium caseinate glues and plastics, although bringing about decided changes in physical and chemical properties of casein, does not produce any characteristic or definite change in molecular structure. Apparently only surface reactions are involved. However, when NaOH was added to casein and the sodium caseinate heated to boiling, a marked change in color appeared. This reaction produced a decided change in the diffraction spacings, as shown in the table below. Apparently there was a tendency for shrinkage and a closer packing of the peptide linkages.

TABLE 2  
*Spacing values for alkaline compounds of casein*

Spacing	Casein NaOH	Casein NH <sub>4</sub> OH	Casein Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub>	Casein Na <sub>2</sub> CO <sub>3</sub>	Casein	NaOH
					Boiled	
d <sub>1</sub>	9.78 Å	9.84 Å	9.78 Å	9.80 Å	9.34 Å	
d <sub>2</sub>	4.61 Å	4.67 Å	4.62 Å	4.60 Å	4.45 Å	

X-ray diffraction patterns for two of the above preparations are illustrated by Figures 4 and 5. Each shows characteristic halos of non-fibrous,

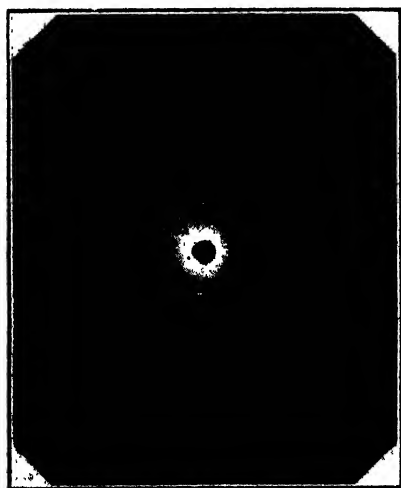


FIG. 4. Sodium caseinate (NaOH + casein).

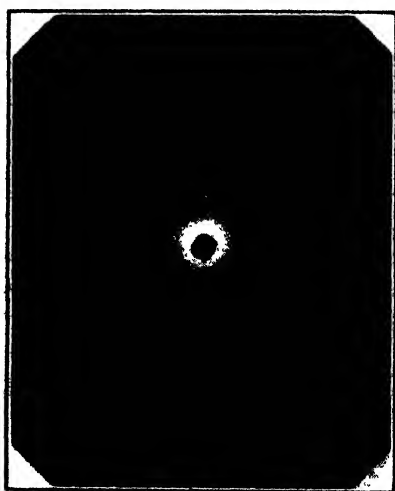


FIG. 5. Boiled sodium caseinate.

non-crystalline proteins. Astbury and co-workers (13) assert that denaturation of proteins brings about a tendency for a fibering of proteins as they are most stable and most insoluble in this condition. According to them, "the denatured state is essentially a fibered one, inasmuch as it always consists of peptide chains often fully extended and aggregated after coagulation, as in fibroin."

Although casein was denatured by boiling in strong alkaline medium, orientation could not be observed. Long threads were produced, dried under tension, and mounted either parallel with, or perpendicular to, the plane of x-rays, but no orientation was noticed. Coagulation of casein by rennin also denatures this protein. However, no fibering or orientation occurs, even though the protein is stretched into long threads.

The reaction of alkalis and acids with casein that brings about a change in the physical properties of casein but not a change in the molecular structure might be explained by assuming that proteins, in general, are amphoteric electrolytes (14). They combine with anions on the acid side of the isoelectric point and with cations on the alkaline side. The isoelectric point of casein is at pH 4.7. In making sodium caseinate the reaction occurs at pH 10, or greater. Under these conditions the reaction would occur at the COOH radical. The sodium caseinate that is formed could then dissociate into a protein anion and a  $\text{Na}^+$  cation. On the acid side of the isoelectric point the amino group of the protein molecule behaves like ammonia in its ability to add an acid. The hydrochloride that is formed could then dissociate into a cation and a  $\text{Cl}^-$  anion. Assuming then that the casein would react as colloidal particles, there are undoubtedly numerous COOH and  $\text{NH}_2$  groups that would react in the manner described, producing changes in physical properties but no profound molecular structural rearrangement due to the reaction.

#### *The Reaction of Casein and Formaldehyde*

The chemical reaction that occurs between casein and formaldehyde is decidedly interesting and, from an industrial viewpoint, highly valuable, for on this reaction is based the production of goods of the casein plastic industry. When a weak solution of formaldehyde (3–5 per cent) is added to a sheet of casein a hardening or denaturation reaction occurs that is solely characteristic of formaldehyde. At first, like the alkali reactions, this appears to be only a surface reaction, since the hardening occurs very shortly after the casein is in contact with the formalin solution. However, the reaction continues and proceeds at a more or less constant rate, according to Henley (15). This investigator worked with serum proteins and found that the formol reaction was of the second order; that is, for a given reaction to occur, the time required is inversely proportional to the original concentration. Although the reaction of formaldehyde on casein rapidly alters

the physical properties of the casein, no marked internal change in structure occurs. However, at the end of five days in 40 per cent formalin a sample of ultra-filter casein showed a new strong diffraction interference that is characteristic of the reaction of formaldehyde and casein.

It was first considered that the presence of the new diffraction interference would aid in interpreting the casein formaldehyde reaction, but further work showed this new line to be due to polymerized formalin that had crystallized in the casein during the reaction (16).

Although formalin produces decided physical changes in casein, it has not been possible by x-ray diffraction analysis, as yet, to show marked molecular structural changes, occurring as a result of the reaction.

#### SUMMARY

By x-ray diffraction analysis an attempt has been made to interpret changes that were produced by the action of acids, alkalis and formaldehyde on casein, as well as, attempting to distinguish differences in casein prepared by various methods.

No marked differences were found in the casein samples prepared by various procedures. Only by vigorous boiling in the presence of NaOH were marked changes in structure observed due to chemical treatment.

The two halos produced by casein upon x-ray diffraction analysis were typical of non-fibrous proteins. The halos were at spacings of approximately 4.6 Å and 9.8 Å which correspond respectively to the vertical distance between layers of polypeptide chains and the longitudinal distance of side chains from the main chain. These values agree well with other non-fibrous proteins that have been subjected to x-ray analysis.

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# AN X-RAY DIFFRACTION ANALYSIS OF CHEDDAR CHEESE

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## INTRODUCTION

The determination of chemical changes occurring in cheddar cheese during ripening has been the subject of research by numerous investigators.

Van Slyke and Hart (1) in 1902 considered the first step in cheese ripening was the peptic digestion of paracasein. They found that as the cheese aged there was an increase in water soluble nitrogenous products.

Later these same investigators (2) studied the individual proteolytic compounds liberated from the aged cheddar cheese. After 214 days at 15.5° C. (60° F.) they found that 15 grams of CO<sub>2</sub> had been liberated, which was .5 per cent of the weight of the fresh cheese. In addition, tyrosine, oxyphenylethylamine, arginine in traces, histidine, lysine, guanidine, and putresine in traces were also identified as hydrolytic products of cheddar cheese ripening.

Kelley (3) showed that the protein of cheddar cheese is hydrolyzed at a fairly uniform rate, and that at 90 days approximately 20 per cent of the protein was water soluble.

Lane and Hammer (4) in studying the rate of protein hydrolysis in cheese made from raw milk, and cheese made from pasteurized milk, concluded that cheese made from raw milk ripened more rapidly as measured by protein hydrolysis.

The purpose of the present investigation was to study the chemical changes occurring in cheddar cheese, both by chemical analysis and by the x-ray diffraction technique.

## EXPERIMENTAL PROCEDURE

### *X-Ray Diffraction Analysis*

The procedure followed in securing the x-ray patterns of the experimental samples has been described in the preceding paper.

The cheese samples used for diffraction analysis were prepared by cutting a thin slice of the cheese approximately .5 mm. thick and then drying it at 37° C. for 12 to 24 hours. The samples were then washed with ether

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\* The data presented in this paper are from a thesis submitted to the Graduate School of the University of Illinois in partial fulfillment of the degree of Doctor of Philosophy, 1937.

to remove the ether soluble material. When cheese samples were not dried, broader and less sharply defined rings were obtained. When the fat was not removed by ether extraction, lines due to liquid fat were obtained.

The pure amino acids which were used for the standard patterns in identifying the amino acids liberated in the cheese were obtained through the courtesy of Professor W. C. Rose, of the Division of Biochemistry, University of Illinois.

#### *Methods for Chemical Analysis of Cheese*

Chemical analyses of the cheeses were made after one day, one week, and at monthly intervals thereafter until the cheese was 180 days old.

The methods used and the procedure followed were much the same as those used by Kelley (3). Hydrogen ion concentration measurements were made, using a L & N Quinhydrone pH Indicator with a saturated Calomel electrode. The technique followed was similar to that which Brown and Price (5) found to be satisfactory.

#### *N-Butyl Alcohol Extraction of Ripened Cheese*

A modification of Dakin's (6) method of n-butyl alcohol extraction of hydrolyzed protein was made to secure mono-amino acids from ripened cheese. The cheese samples after being grated to shreds were extracted with petroleum ether until practically fat free. Two hundred to five hundred grams of cheese were extracted four times with one liter of hydrated n-butyl alcohol. The cheese and butyl alcohol were mixed by shaking on a mechanical shaker one to two hours for each extraction. The alcohol extract was filtered and then evaporated under reduced pressure at 30° C. to about one-fourth its volume. By that time the mono-amino acids had crystallized in the dry butyl alcohol so that they could be filtered off, dried, weighed, and analyzed by x-ray diffraction methods.

#### *Manufacturing Procedure for Cheese Used for Chemical and X-ray Analysis*

The manufacturing procedure for the two lots of cheddar cheese used in the ripening study was varied in such a way as to produce one lot which would cure slowly and the other lot which would ripen rapidly. This was done by varying the amount and rate of acid development. Lot 1115 was the slow ripening cheese and lot 1120 was the rapid curing cheese. Both lots were made from pasteurized milk of low bacterial count. The manufacturing data for the cheese is listed in Table 1.

During the manufacturing process samples of the cheese were taken for x-ray analysis. Also at definite intervals throughout the ripening period, the cheese was subjected to x-ray examination and chemical analysis in order to correlate the changes in the patterns that occurred with the rate

TABLE 1  
*Manufacturing data for experimental cheese*

	Lot 1115	Lot 1120
Pounds of milk . . . . .	891	339
Weight of cheese made . . . . .	91.25	35.5
Per cent of starter used . . . . .	1.5	2.25
Incubation time at 86° F. . . . .	90 min.	150 min.
Titrateable acidity of whey at curd cutting . . . . .	.09%	.145%
pH of whey at cutting . . . . .	6.74	6.32
pH of curd at cutting . . . . .	6.75	6.28
Titrateable acidity of whey at dipping . . . . .	.12%	.29%
pH of whey at dipping . . . . .	6.41	6.01
pH of curd at dipping . . . . .	6.38	5.54
Titrateable acidity of whey at milling . . . . .	.45%	.8%
pH of whey at milling . . . . .	5.57	5.13
pH of curd at milling . . . . .	5.57	5.23
pH of curd at dressing . . . . .	5.57	5.23
pH of curd after 24 hours . . . . .	5.24	5.04
Per cent of water in freshly made cheese . . . . .	36.9	35.5

of protein hydrolysis. Ripening of the cheese was carried on in a cold storage room, the temperature of which was controlled at  $10^{\circ}\text{C.} \pm 1.5^{\circ}\text{C.}$

#### DISCUSSION OF EXPERIMENTAL RESULTS

##### *Chemical Analysis*

Since the data obtained by chemical analysis is comparable to the work of previous investigators, a discussion of these results will be omitted.

##### *Analysis of Cheese by X-Ray Diffraction Methods*

The manner in which x-rays have been used in this part of the study is comparable to qualitative in contrast to quantitative analysis. A series of standard x-ray diffraction patterns of the amino acids which occur in casein were made and the spacings calculated. The calculated spacings of the cheese patterns and the spacings of the mono-amino acid extract from the cheese were then correlated with the spacing values of the standard patterns of the amino acids.

As has been shown in the previous paper the diffraction pattern of casein upon x-ray analysis shows two halos at calculated spacings of 4.6 Å and 9.8 Å, which are similar to other proteins as found by Astbury (7) and others (8). The outer halo at 4.6 Å represents the perpendicular distance between the layers of polypeptide chains. Astbury has called this the "backbone" spacing. The inner halo at approximately 9.8 Å represents the length of the polypeptide side chains.

Theoretically, in terms of the dimensions of the  $\text{CH}_2$  and  $\text{CONH}$  groups, the packing of peptide chains in the two planes should be given by 4.64 Å

and 9.68 Å, provided the amino acids of the chain are on the average of the length of valine (9). The casein values as determined, therefore, agree well with the theoretical prediction.

In order to secure the desired information about cheese ripening, diffraction patterns of the two lots, 1115 and 1120, were secured at regular intervals. Coagulation of casein by rennin and the formation of paracasein are fundamental to the process of making cheese. Accompanying this coagulation reaction marked changes occur not only in the chemical properties of the casein but in the physical as well. It becomes elastic and while warm will stretch into long strings, which shatter and break readily on cooling. However, the same x-ray diffraction patterns were obtained for casein, paracasein, and stretched paracasein. Proteins, such as keratin, and gelatin, when stretched show a marked change in structure, with a decided orientation of diffracting groups. Apparently stretching of paracasein is not a true stretching comparable to that which occurs when tension is placed on rubber or muscle fiber, but is more similar to a plastic flow.

Although the patterns of fresh cheese or paracasein are the same as the patterns for any type of casein, the x-ray pattern changes quite rapidly under certain conditions as the cheese ripens. The changes that occur may be briefly described as follows:

First, the outer halo gives way to two sharply defined rings at the inside and outside of the halo. The spacings are at approximately 4.6 Å and

TABLE 2

*Calculated values for the spacings determined from the diffraction patterns, cheese 1115*

Sample treatment				Ave. value for "d" spacings during period	Ave. "d" spacings for 30 other samples
1115 plain	1115 plain	1115 plain before H <sub>2</sub> O extract	1115 after H <sub>2</sub> O extract		
Date of exposure					
11-16-35	11-20-35	5-31-36	5-31-36		
Age of cheese in days					
1	5	196	196		
9.7	9.78	9.53	9.90	9.80	9.73
	7.58	7.60	7.61	7.62	7.55
4.6	4.55	4.55	4.57	4.58	4.60
	4.14	4.19	4.23	4.22	4.23
		3.82	3.8	3.82	3.83
					3.40
	3.01 S*	3.07 S	3.03 S	3.04	3.05
	2.89 S	2.92 S	2.91 S	2.91	2.92
	2.57 S	2.64 S	2.60 S	2.60	2.61
				2.42	2.43

\* S = Strong intensity.

4.23 Å. The inner halo is retained, but a new spacing appears at 7.6 Å near the outer edge of this small inner halo. The above changes are the first to appear in the ripening process. As the period progresses other spacings are brought out in the x-ray diffraction pattern. These spacings have been calculated to be at 3.85 Å, 3.42 Å, 3.08 Å, 2.92 Å, 2.61 Å and 2.43 Å. The typical patterns of unaged and aged cheeses are illustrated by Figures 1 and 2. Figure 3 shows two patterns on the same film and illustrates clearly the sharp contrast between the diffraction rings of the unaged and the aged cheese samples.

In Tables 2 and 3 are listed the calculated values for the spacings determined from the diffraction patterns which were made throughout the ripening period of the cheese samples.

The most variable spacing in the cheese is the one which represents the length of the peptide side chain linkage. This spacing, in lot 1115, varied from 9.4 Å up to 10.1 Å. However, this value is more constant between the 1115 samples and the total variation is not as great in this lot as in lot 1120. For in this group of samples, the spacing values varied from 9.12 Å to 10.5 Å. Clark and Schaad (10) consider that the different values found for this spacing in other proteins are due to the difference in the amount of water retained by the protein. It is true that when a sample of moist cheese is ex-

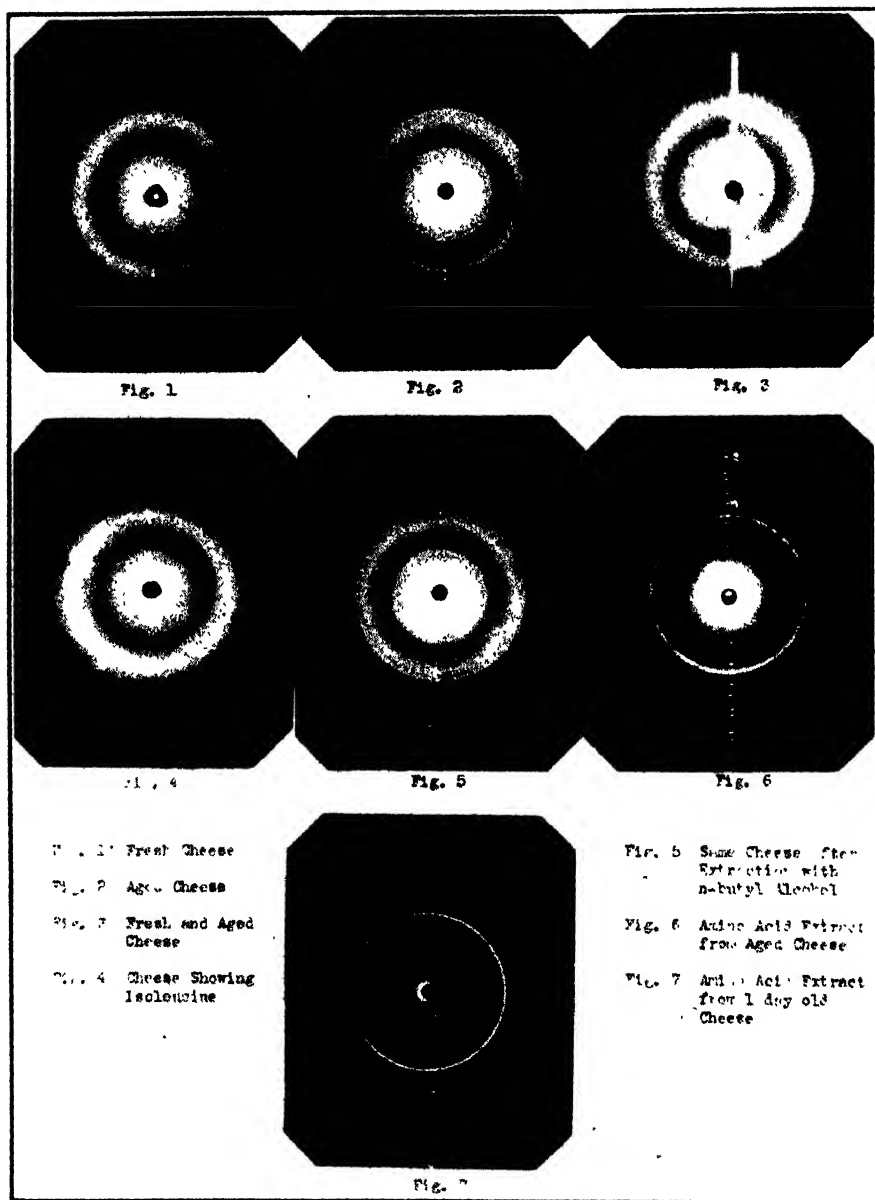
TABLE 3

*Calculated values for the spacings determined from the diffraction patterns, cheese 1120*

Sample treatment				Ave. value for "d" spacings during period	Ave. "d" spacings for 30 other samples
1120 Plain	1120 Plain	1120 before H <sub>2</sub> O extract	1120 after H <sub>2</sub> O extract		
Date of exposure					
11-21-35	11-26-35	5-20-36	5-20-36		
Age of cheese in days					
1	6	181	181		
9.5	9.12	9.95	9.9	9.8	9.73
	7.68	7.65	7.65	7.66	7.55
4.61	4.65	4.7	4.62	4.63	4.60
4.3 V.F.*	4.2	4.3	4.29	4.28	4.23
			3.86	3.83	3.83
					3.40
	3.03 V.F.	3.07 S	3.07 S	3.04	3.05
	2.89 V.F.	2.94 S	2.94 S	2.90	2.92
	2.60 V.F.			2.60	2.61
					2.43

\* V.F. = Very faint intensity.

S. = Strong intensity.



amined by x-rays this halo becomes indistinct and decreases in diameter, a change which indicates a larger "d" spacing.

The values for the other spacings do not exhibit the variability of the first spacing, but are surprisingly uniform. The interesting observations about the remaining spacings are the time of occurrence and the intensity of diffraction lines. The question arises as to the significance of these new spacing values in the cheese paracasein. Three possibilities might be advanced to

explain their presence. First, since cheese ripening represents an hydrolysis of protein and a liberation of amino acids, the diffraction lines may be due to amino acids. Second, the mineral constituents of the cheese may have crystallized and the characteristic pattern may be the result of the diffraction of the x-rays by the mineral salts. Third, the minerals or the amino acids may be combined with the paracasein in such a manner as to produce a crystalline pattern but still not exist as pure crystalline substances in the cheese.

If the characteristic pattern of cheese paracasein were due to free amino acids, then this pattern should be changed by the extraction of the cheese in water or hydrated n-butyl alcohol. The alcohol is a solvent for the mono-amino acids and the di-amino acids are also slightly soluble in and extracted by the n-butyl alcohol. However, the spacing values of the cheese protein are not changed by extraction of the cheese in either this solvent or water. Diffraction patterns were made of di-calcium phosphate, tri-calcium phosphate, calcium lactate and mixtures of these, but the spacing values of the cheese could not be reproduced.

On the other hand when the cheese was extracted in 10 per cent HCl, which is a solvent for both minerals and amino acids, the characteristic pattern of the cheese disappeared and the pattern reverted to one of two halos characteristic of the plain protein at the beginning of the ripening period.

In Table 4 are listed the amino acids that could possibly account for the spacing values. However, because of the similarity in structure of the amino acids the "d" spacings are approximately the same. Hence, the amino acids could not be identified specifically by these values although some weight might be attached to the fact that the spacings for isoleucine, leucine, valine, OH-proline, proline, aspartic acid and tyrosine occur most often.

#### *Extraction of Cheese with Hydrated n-Butyl Alcohol*

Dakin (6) proposed a method for the separation of amino acids into groups after the protein had been hydrolyzed by sulphuric acid. He considered that the mono-amino acids were soluble in n-butyl alcohol and could, therefore, be separated from the di-amino and di-carboxylic acids, which were supposedly insoluble in that reagent. Later investigators, notably Rose and his students, have shown that the alcohol extract does contain considerable amounts of the di-amino acids as well as proline and hydroxy prolines. However, Rose and his associates (11) were led to their discovery of the new essential amino acid, threonine, through the use of the butyl alcohol extraction method in isolating amino acids for feeding experiments.

The separation of amino acids from their mixtures is decidedly difficult because of the similarity of properties of the various amino acids. It was decided, therefore, to determine the adaptability of x-ray analysis to the identification of amino acids in the mixtures that would likely occur in the alcohol extract from the cheese.

TABLE 4

"d" spacings in Å found in cheese	"d" spacings in Å in amino acids
7.62—(1115)	7.61 Histidine—HCl
7.66—(1120)	
4.58—(1115)	4.64 Methionine
4.63—(1120)	4.60 Arginine
	4.56 Valine
	4.54 Isoleucine
4.22—(1115)	4.22 Leucine
4.28—(1120)	4.23 Aspartic acid
	4.26 Hydroxyproline
	4.27 Phenylalanine
3.82—(1115)	3.80 Histidine
3.82—(1120)	3.84 Isoleucine
	3.84 Phenylalanine
	3.86 Aspartic acid
	3.87 Proline
	3.87 Alanine
3.42	3.4 Valine
	3.4 Tyrosine
	3.4 Proline
	3.4 Threonine
	3.45 Alanine
3.04—(1115)	3.08 Glutamic acid
3.04—(1120)	3.08 Phenylalanine
	3.09 Histidine—HCl
2.91—(1115)	2.90 Isoleucine
2.90—(1120)	2.93 Tyrosine
	2.95 Leucine
	3.00 Valine
2.60—(1115)	2.61 Isoleucine
2.60—(1120)	2.61 OH—proline
	2.62 Proline
	2.62 Norleucine
	2.63 Valine
	2.63 Arginine
	2.64 Tyrosine
2.43—(1115)	2.40 Leucine
	2.41 Isoleucine
	2.41 Methionine
	2.41 Histidine—HCl
	2.41 Threonine
	2.46 Valine

Standard patterns of the amino acids occurring in casein were made, as well as patterns of physical mixtures of some of them. The spacing values for the various mixtures are illustrated in Table 5. As can be seen, when there is a mixture in equal amounts of only two amino acids (namely, leucine and isoleucine), it is relatively simple to identify them. However, when tryptophane is added to the mixture another problem arises, that of destructive interferences. There is one spacing value, 17.1 Å, that is specific for

TABLE 5

Standard spacing values for isoleucine and leucine (1 to 1) mixture			Standard spacing values for leucine, isoleucine, and tryptophane (1 to 1 to 1)		
Spacing on pattern of mixture in A	Standard pattern value in A		Spacing on pattern of mixture in A	Standard pattern value in A	
13.03	Isoleucine	13.03	17.1	Tryptophane	17.1
6.4	Isoleucine	6.52	13.02	Isoleucine	13.03
5.51	Isoleucine	5.51	6.5	Isoleucine	6.52
4.96	Leucine and Isoleucine	4.98	5.55	Isoleucine	5.51
			5.02	Leucine and Isoleucine	4.98
4.65	Leucine	4.65	4.68	Leucine	4.65
4.18	Isoleucine	4.18			
4.01	Leucine and Isoleucine	4.00	4.24	Leucine	4.22
			4.08	Leucine	4.00
3.84	Isoleucine	3.84		Isoleucine	4.02
3.47	Leucine	3.48	3.88	Isoleucine	3.88
			3.52	Leucine	3.48
3.24	Isoleucine	3.24		Isoleucine	3.50
3.16	Leucine	3.17	3.22	Isoleucine	3.24
2.95	Leucine	2.95	3.02	Isoleucine	3.00
			2.92	Leucine	2.95
2.89	Isoleucine	2.89		Isoleucine	2.89
			2.79	Leucine	2.77
2.77	Leucine	2.77		Isoleucine	2.76
2.67	Isoleucine	2.67	2.62	Isoleucine	2.60
2.60	Isoleucine	2.60	2.52	Isoleucine	2.51
			2.42	Isoleucine	2.41
2.50	Isoleucine	2.51		Tryptophane	2.45
2.40	Leucine	2.41			

tryptophane and if this spacing had not appeared, tryptophane could not have been identified as being part of the mixture. This indicates that qualitative chemical tests should also be made on mixtures so as to serve as confirmatory tests. The observation that the spacings for tryptophane were absent is significant because it held true in the unknown cheese extracts, when even one characteristic spacing value for tryptophane did not appear. However, the Hopkin's Cole test showed tryptophane to be present in all the extracts except the one from cheese one day old.

In the first part of Table 6 there is listed the values for the spacings as found in the extracted material from a cheese one day old. The spacing values are, without much doubt, due entirely to leucine and isoleucine. Apparently then, these two amino acids are liberated fairly rapidly in the hydrolysis of casein during cheese ripening. There was a negative test for tyrosine, although one would expect tyrosine to be liberated just as early as leucine and isoleucine. Evidently this was not the case. In fact, in testing all of the cheese extract samples, a much stronger test was obtained for tryptophane than for tyrosine, providing tryptophane was present at all. Womack and Rose (12) have found that leucine and isoleucine crystallize at

TABLE 6

Identification of amino acids in butyl alcohol extract from cheese					
First extraction from cheese 426 age, 1 day			First extraction from cheese 2235 age, 4 months		
Value for mixture	Standard pattern value		Value for mixture	Standard pattern value	
13.9	Leucine	14.2	13.95	Leucine	14.2
5.25	Leucine	5.24	9.35	Lysine	9.05
4.55	Isoleucine	4.54	6.45	Tyrosine	6.55
	OH—Proline	4.52		Isoleucine	6.53
4.16	Isoleucine	4.15	4.75	Phenylalanine	4.75
3.86	Isoleucine	3.84		Leucine	4.72
	Phenylalanine	3.84	3.93	OH—Proline	4.72
3.69	Isoleucine	3.68		Tryptophane	3.95
	Histidine	3.68	3.34	Leucine	4.00
2.55	Leucine	2.55	3.23	Phenylalanine	3.32
2.34	Leucine	2.30		Methionine	3.36
				Isoleucine	3.24
				Methionine	3.23
				Proline	3.21
				OH—Proline	3.22
			3.10	Phenylalanine	3.08
				Histidine—HCl	3.09
			2.91	Arginine	3.10
				Isoleucine	2.91
			2.47	Tyrosine	2.93
				Isoleucine	2.41
				Methionine	2.41
				Histidine—HCl	2.41
Negative—Hopkins Cole			Positive—Hopkins Cole for Tryptophane		
Negative—Millon test for Tyrosine			Sl. positive for Tyrosine by Millon's reagent		
Negative—unoxidized sulphur test			Negative for unoxidized sulphur		

the same time when in a mixture. X-ray analysis can detect the presence of either of these when they exist in appreciable amounts in a mixture, as is shown by Table 5.

Figures 4 and 5 illustrate that leucine can be extracted from an aged cheese sample. The small inner ring on the cheese pattern of Figure 4 indicates a spacing which is specific for leucine. Upon extraction of the cheese by n-butyl alcohol, this ring disappeared. (See Figure 5.) However, the x-ray diffraction pattern of the amino acid extract shows that it is present there. (Figure 6.)

In part two of Table 6 are shown the spacing values for a cheese sample four months old. Lysine might be present, in addition to leucine, isoleucine, tryptophane, and tyrosine.

Table 7 lists the values for the extract from a cheese sample approximately eighteen months old. The presence of leucine and isoleucine are clearly illustrated. Tyrosine and tryptophane were shown to be present by the characteristic color tests for amino acids that were applied to the crystalline material.

TABLE 7

Identification of amino acids in butyl alcohol extract from cheese					
First extraction from cheese 12274 age, 15 months			First extraction from commercial cheese age, approximately 18 months		
Value for mixture	Standard pattern value		Value for mixture	Standard pattern value	
13.9	Leucine	14.2	14.3	Leucine	14.2
7.31	Aspartic acid	7.42	7.31	Aspartic acid	7.42
5.21	Leucine	5.24	4.99	Isoleucine	4.99
				Leucine	4.98
4.60	Isoleucine	4.56		Tyrosine	4.90
	Arginine	4.60			
			4.56	Isoleucine	4.54
4.00	Leucine	4.00		Proline	4.53
	Isoleucine	4.02		OH—Proline	4.52
	Phenylalanine	3.99			
			4.07	Aspartic acid	4.06
3.65	Isoleucine	3.68		Isoleucine	4.03
	Serine	3.66		Tyrosine	4.09
	Phenylalanine	3.64		Threonine	4.09
	OH—Proline	3.64			
			3.58	Tyrosine	3.59
3.25	Isoleucine	3.24			
	Proline	3.21	3.23	Isoleucine	3.24
	OH—Proline	3.22		OH—Proline	3.22
				Methionine	3.22
2.97	Leucine	2.96			
	Arginine	2.97	3.12	Leucine	3.15
	Lysine	2.95		Aspartic acid	3.13
				Lysine	3.13
2.82	Leucine	2.82			
	Threonine	2.82	2.80	Leucine	2.82
				Proline	2.80
2.40	Leucine	2.40			
	Isoleucine	2.41			
	Arginine	2.40			
	Threonine	2.40			
Positive—Hopkins Cole test			Positive—Hopkins Cole test		
Sl. positive—Millon's test			Positive—Millon's test		

Figures 6 and 7 show the x-ray diffraction patterns of the amino acid extracts from two different samples of cheese. As one can observe, there is a difference in the patterns, indicating that the crystalline extracts were not identical in composition in all respects. The calculations of the spacing values listed in the Tables 6 and 7 also show that this is true.

#### *A Long Interplanar Spacing in Cheese*

Clark and Schaad (10) have shown that certain proteins; namely, nerve tissue and intestinal wall collagen, contain certain long spacings that can be isolated by a special technique. Spacings varying from 70 Å up to 400 Å have been found in intestinal wall collagen when using a vacuum camera and a distance of 20 cm. from specimen to film. The long spacing which was found in cheese, but which was absent from casein, was calculated to be

39.7 Å. However, the nonappearance of the long spacing interference in pure casein is still unaccounted for.

Svedberg (13) calculated the radius of the casein particle, assuming that it was spherical, to be 41.7 Å to 59.94 Å. The two values, 41.7 Å for casein and 39.7 Å for cheese, are of the same order of magnitude. Therefore, it is possible that this spacing represents the radius of the casein particle, or the maximum length of the side chain spacing from the main chain. If this be true, the size of the casein particle which has been arrived at by another method agrees well with that determined by Svedberg.

#### SUMMARY

An attempt has been made to follow the changes which occur during the ripening of cheddar cheese by x-ray diffraction analysis.

The diffraction pattern of the cheese protein after aging is markedly different from the pattern of the fresh cheese. The pattern appears to be due to crystalline material, although the identification of the material has not been made as yet.

Standard patterns were made and "d" spacing values were calculated for the amino acids occurring in casein in order to determine whether the characteristic pattern was due to an amino acid of low solubility.

By the extraction of the aged cheese in hydrated n-butyl alcohol, amino acids were extracted from the cheese and identified. Leucine and isoleucine were obtained from cheese 24 hours after being made. Tyrosine, and probably tryptophane were also identified in the extract obtained from ripened cheese. A long interplanar spacing of 39.7 Å was obtained from cheese protein. This spacing represents the length of a side chain from the main chain and corresponds closely with the radius of the casein particle which Svedberg determined to be 41.7 Å to 59.9 Å

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## JUDGING SWEET CREAM

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The more general application of scientific methods in the production, manufacture and merchandising of dairy products during the last quarter century has very markedly improved the quality of the goods which the industry offers to the consumer. Whether one contemplates large scale production such as is realized in the thrice-daily milking of 1600 cows on one large eastern farm, or a city pasteurizing plant processing 400,000 pounds or more of milk daily, or the nation-wide marketing methods of the larger dairy manufacturers, one is everywhere impressed by the emphasis laid on the fine, healthful quality of the product. This growing stress on quality is making the consumer more discriminating in the selection of brands or sources of supply and would seem to require that the industry reexamine carefully the methods of measuring quality which are currently employed for this purpose.

In the past little attention has been given to the evaluation of the quality of sweet fluid cream offered to the public for use in coffee, with fruits and cereals, or as a whipped cream. It is true that the U. S. Department of Agriculture some years ago adopted, with the approval of the American Dairy Science Association, a score card for the alternative judging of milk or cream. (1) This score card allotted 40 points to physical characteristics, divided into 25 points for flavor, 10 for sediment and 5 for package, and 60 points to laboratory-tested qualities, of which 45 depended on total plate counts of microorganisms and 15 on titratable acidity or, alternatively, the temperature of "street" samples.

Regardless of opinion as to whether such a weighting of the several factors is justifiable or desirable in judging fluid milk as marketed today, there can be little doubt that sweet fluid cream should be rated on a different basis. In the first place, the volume of milk now being sold in the form of sweet cream is sufficient to warrant separate consideration for this product, and, in the second place, the qualities which determine the marketability of cream differ considerably from those which determine the marketability of milk.

Statistics compiled by the Federal government in several of the large eastern markets (2) show that at least 40 per cent of the total fluid milk volume consumed in these markets is distributed in the form of fluid cream. This relationship may not hold exactly in all sections of the United States, but is probably applicable to most urban centers where the bulk of sweet cream sales occurs.

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It is evident that sweet cream occupies, both from the dollar value and from the volume of milk required for its manufacture a very high place in the economics of fluid milk distribution.

The question arises as to what characterizes high quality in fluid cream. Certainly flavor is of prime importance, since a large proportion of the fluid cream sold is consumed on fruits, cereals, cake, etc., which do not mask the flavor of the cream itself. Off-flavors in cream are a primary source of customer complaints. For this reason the distributor of fluid cream is very conscious of the necessity of excellence of flavor. It would seem reasonable in scoring cream that flavor should be given as much consideration as in scoring butter, where forty-five points out of 100 are allotted to flavor.

"Pourability" or consistency, or viscosity, is another factor of prime importance in the eyes of the customer. Being aware of the general association of increased body with higher fat content, the housewife suspects a thin-pouring cream as being low in butterfat and is likely to enter a complaint with her distributor.

Whipping quality is probably more troublesome today to the milk distributor than any other characteristic of fluid cream. Many resort to the costly expedient of increasing the fat content to satisfy their customers as to whipping quality. Others add "viscogen," lecithin or skim milk solids, practices which are illegal in many areas and which are eschewed by leaders in the industry as partaking of adulteration.

Cream "plug," giving rise to oiliness in coffee, is a frequent defect of fluid cream, while the separation of a serum layer in bottled cream is a very common occurrence in the lower fat ranges. Sediment, showing lack of cleanliness in production and handling, must always be considered in judging quality. The appearance of the container is a factor which is being emphasized in all food merchandising today and should be accorded some weight in any rating of quality of a packaged food.

The present score card for milk and cream allots 45 points to total bacterial counts, giving a cream with a plate count of 100,000 organisms per ml. a zero score in this bracket. This emphasis on bacterial content seems entirely out of proportion to its importance today. Under modern conditions of city milk regulation and inspection the general condition of fluid cream from a bacteriological standpoint is quite satisfactory. The public is adequately protected from pathogenic organisms by pasteurization requirements, and the subsequent protection of the product against temperature change, so that total counts are of importance mainly because of the effect of putrefactive and acid types of organisms on flavor and keeping quality. If samples are judged twenty-four hours after processing, the effect of objectionable high counts will probably be manifested in off-flavors. For these reasons we believe that 15 points gives sufficient weight to bac-

terial counts, penalizing a sample one point for each 10,000 per ml. count above 10,000. By this means any cream containing in excess of 160,000 bacteria per ml. would be penalized 15 points.

Acidity is of importance as it affects keeping quality and flavor. Prevailing conditions of producing and processing sweet cream result in low acidities as well as low bacterial counts at time of packaging. Since subsequent carelessness in handling may lead to bacterial growth and developed acidity, a small penalty for acidity increase would appear to serve for

TABLE I  
*Cream Score Card*

Sample No. _____	Butterfat Content _____	Date _____
Judge after 24 hrs. aging Enter observed data—Underline defects		Perfect score    Points deduct.    Score allow.
<b>FLAVOR:</b> Exc. (34-40) No definite criticism		40 _____
Good (28-33) Very slight feed, sl. cooked		_____
Fair (23-27) Cooked, feed, sl. unclean or metall.		_____
Poor (13-22) Metallic, str. feed, unclean, sl. acid, sl. rancid or tallowy		_____
Bad (0-12) Sour, foreign, racid, tallowy, putrid		_____
<b>BODY:</b> <i>Light Cream Score</i> <i>WHIPPING</i> 20		
—sec. Exc. (92 to 100%) 18-20	Sec. to whip _____	
—sec. Fair (72-92%) 13-17	4 points for Exc. _____	
—sec. Poor (48 to 72%) 7-12	Cm. Penetr. _____	Body _____
<i>Heavy Cream Score</i>		
—sec. Exc. (as above) 9-10	2 points for Exc. _____	
—sec. Fair (" " " " ) 6- 8	Volume Incr. _____%	
—sec. Poor (" " " " ) 2- 5	2 points for Exc. _____	
Refer to tables for Excellent standards    2 points for Exc.    Total (20 max) _____		
N.B. If whipping tests not made, score <i>Heavy Cream</i> Body same scale as light.		
<b>BACTERIA</b> 10,000 or less considered perfect		15
<b>COUNT:</b> For higher counts deduct 1 point per 10,000 additional		
_____ per ml.		
<b>CREAM PLUG:</b> Deduct: 0-1 Sl., soft, foamy plug		5
1-2 Distinct soft plug		
2-3 Buttery plug		
4-5 Leathery plug		
<b>SERUM SEPARATION:</b> Deduct 1 point for each 1/16" serum		5
<b>ACIDITY:</b> Deduct 1.0 for each 0.1% acid above normal acidity for cream of specified fat content		5
<b>SEDIMENT:</b> Penalize in proportion to specks or dirt		5
<b>PACKAGE:</b> Dirty bottle—deduct 0.5-1.0		5
Bottle etched or chipped—deduct 0.25-1.0		
Slack fill—deduct 1.0 for each 1/2" short		
Unprotected lip—deduct up to 1.0 point		
Poor cap condition—deduct up to 1.0		
<b>COMMENTS:</b>		<b>TOTAL SCORE</b> _____
		Scorer _____

quality maintenance. Actual souring would be severely injurious to good flavor and would bring a correspondingly heavy penalty in that part of the score.

With these ideas in mind it appeared desirable to propose a new method for the evaluation of the quality of fluid cream, one which would reflect more accurately the various factors entering into its determination and one which might be better adapted to conditions in the industry today. We believe that this subject merits thoughtful consideration by dairy science workers, both in the academic and in the industrial fields. In the following scheme (Table I) which has had a year's practical trial in a number of fluid milk plants with favorable endorsement, 40 points are allotted to flavor, 20 points to body or body and whipping quality, 15 points to bacterial counts, 5 points each to "plug," serum separation, acidity, sediment and package.

#### COMMENTS ON SCORE CARD

Quart samples of cream are required to complete all determinations required by the score card. All measurements should be made on creams 24 hours after bottling.

The off-flavors referred to are so well-recognized and defined as to require no elaboration here. Any particular criticisms may be indicated by underlining the appropriate terms. Body and whipping quality present more of a problem in evaluation. Twenty points are suggested for this group, all of which are allotted to body for creams of lower fat content, but are divided between the two for those classified as "whipping creams." Henning (3) has discussed the measurement of the viscosity of cream by means of the MacMichael viscometer and the Borden Flow Meter. It is proposed to use these instruments alternatively, scoring this quality according to the reading obtained. In Table II there is suggested a range of

TABLE II  
*Viscosity of fluid cream at 15.5° C. (60° F.)*

% B.F.	Body flow time (seconds)		Viscosity (centipoises)		% B.F.	Body flow time (seconds)		Viscosity (centipoises)	
	Exc.	Poor	Exc.	Poor		Exc.	Poor	Exc.	Poor
18	38	- 26	2	- 1.75	32	75	- 42	18	- 6
20	40	- 28	7	- 2.0	34	90	- 48	22	- 12
22	44	- 30	8	- 2.25	36	120	- 60	29	- 14
24	48	- 32	11	- 2.5	38	170	- 85	38	- 22
26	52	- 34	12	- 2.5	40	230	- 115	47	- 25
28	58	- 36	14	- 3.	42	290	- 145	60	- 34
30	65	- 38	15	- 4.	44	360	- 180	65	- 39

expected values which experience indicates to be reasonable for pasteurized cream aged 24 hours in the original package.

The numerical score to be assigned to a cream of a given fat content is determined by the percentage of the "Excellent" value obtained for the particular sample, as indicated in the score card.

#### WHIPPING QUALITY

If whipping creams are to be rated for whipping quality, it is essential to employ a standard method which is simple, easily reproducible and employs inexpensive equipment. Several procedures have been described by investigators (4, 5, 6, 7, 8, 9, 10). It is believed the following one well meets the requirements laid down:

##### *Equipment*

1. "Duplex" whipper and bowl.\*
2. 250 and 100 ml. graduates.
3. 600 ml. beakers.
4. 40 gm. Penetrometer (see Figure 1).
5. 90 mm. 60° glass funnels.
6. 1" wire screen discs (20 mesh per inch)
7. Thermometer, grease pencil, spatula, stop watch.

##### *Method*

While the score card should normally be applied to samples 24 hours after pasteurization, it may happen that the bottle of cream has warmed up somewhat before examination. For this reason it is desirable to make certain that the sample has been held below 7° C. (44.6° F.) for at least two hours prior to testing. Cool whipper and bowl to same temperature before making test.

1. Measure 200 ml. of cream into bowl. Adjust temperature to 7° C. With uniform speed of whipper (120 R. P. M.), record *whipping time* in seconds to whip cream to maximum stiffness. Avoid over-whipping.

2. Transfer whipped cream to a 600 ml. beaker, gently tap bottom of beaker on palm of hand to pack and level the whipped cream, mark the surface level with grease pencil for making *volume determinations* later.

3. Determine *stiffness of whip* with penetrometer. Tighten glass sleeve of instrument in burette clamp at a convenient height. Raise penetrometer with fingers so beaker may be set beneath it. Lower the stem until disc just rests on surface of the whipped cream. Release fingers after noting reading on stem opposite upper edge of sleeve. Record penetration in cms./min.

4. Transfer whipped cream to glass funnel fitted with wire disc as a support for cream. Place graduated cylinder under funnel to collect leakage. Expose samples for 2 hours at room temperature (20–22° C.) Record *leakage* in milliliters for 2 hour period.

5. After removing whipped cream from beaker, fill to whipped cream level with water and measure for *volume*. Record the percentage increase in volume.

\* Manufactured by V. V. Vale Corporation, Oak Park, Illinois.

Ten points are allotted to whipping quality, divided between stiffness, volume increase, leakage and time to whip. In Table III is suggested a tentative criterion for evaluation of these factors.

TABLE III  
*Whipping quality of cream*

	Excellent	Fair	Poor
Time to whip . . . . .	Under 40 seconds	40-90 seconds	Over 90 secs.
Stiffness . . . . .	No penetration	0-2.0 cm./min.	Instant penetration
Volume increase . . . . .	90-110%	70-90%	70% or less
Leakage . . . . .	< 4 ml.	4-14 ml.	14-20 ml.

Blank spaces are provided on the score card for recording the actual measurements in determining body and whipping quality.

#### BACTERIA COUNT

Total plate counts are determined by the A. P. H. A. standard method for milk. If less than 10,000 per ml. the sample receives a full score of 15. One point is deducted for each ten thousand or fraction thereof above ten thousand per ml.

#### SERUM AND PLUG

Serum separation is easily recognized by the distinct bluish layer in the bottom of the bottle, which frequently occurs following storage. "Plug" is best observed through removal of the bottle cap. It is objectionable to the consumer because it hinders pouring and causes oiliness on the surface of hot coffee. The deductions proposed in the score card are self-explanatory.

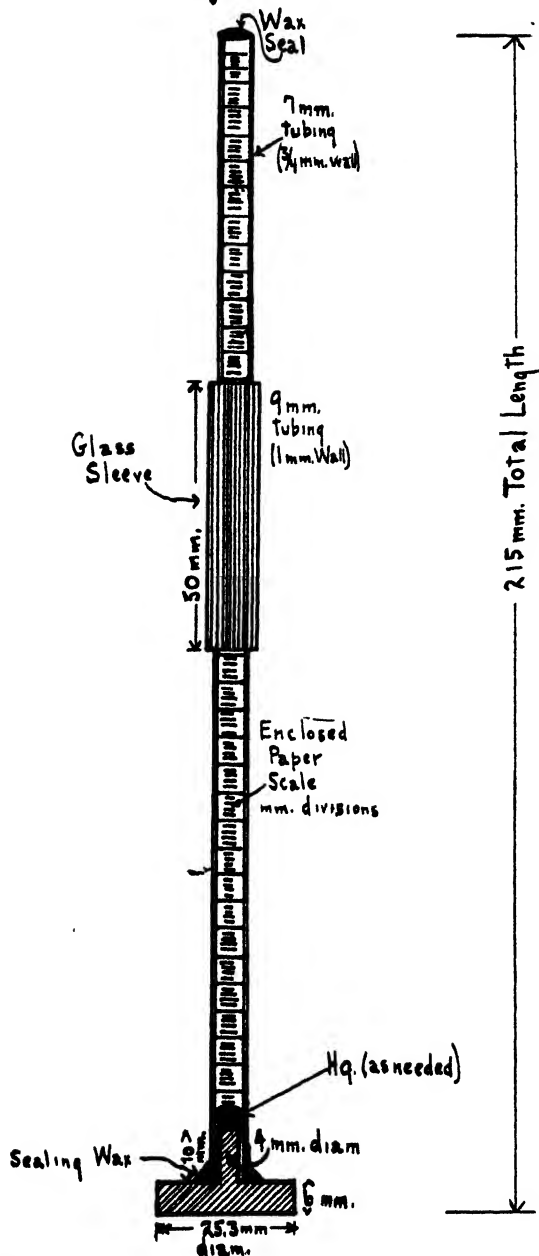
#### ACIDITY

The acidity should be determined by titration of a 9 cc. portion of the cream diluted with 9 cc. of distilled water, using N/10 alkali and 3 drops of phenolphthalein indicator. Since the acidity of cream depends upon the serum present, it will vary inversely with the fat content of the cream. Hence, limits for permissible acidity are determined by calculating the observed titratable acidity value to the basis of the serum alone. A reading of 0.11 per cent in 40 per cent cream is equivalent to 0.18 per cent in skim milk and represents the maximum permissible for a perfect score. One of over 0.14 per cent in 40 per cent cream, equivalent to 0.23 per cent in the serum portion, would be scored zero. Corresponding limits for 20 per cent fat cream would be 0.145 per cent and 0.185 per cent acidity. For intermediate fat contents similarly calculated limits should be used.

#### SEDIMENT AND PACKAGE

Deductions for sediment are based upon the appearance of filter discs obtained by filtering pint samples of cream. A method for preparing such

Fig. 1



Penetrometer for Whipped Cream

discs has been proposed (11) which consists of diluting one pint of cream with an equal volume of filtered hot water, and filtering it through one of the standard milk sediment testers at a temperature of about 50° C. Deductions for sediment are made in proportion to the dirt on the disc.

The defects in package are the same as those now recognized in scoring milk and cream.

#### PLANT APPLICATION

The proposed method for determining the quality of sweet cream has received practical trial in the laboratories of a number of plants. Some have used the score card as a daily routine procedure, particularly for light cream. Others have employed it in periodical surveys of samples distributed in given local areas. The results of the examination of several hundred creams of different fat contents have been recorded. The scores ranged from a maximum of 98 to a minimum of 48, indicating the potential value of such a numerical expression of measurable characteristics as a yardstick by which high quality can be maintained in day-to-day production. Comments made by individual workers indicate their appreciation of the value of such a written record. The score card in its present form appears to offer a convenient, workable tool for routine laboratory control purposes as well as an excellent technique for comparing the quality of a group of cream samples as to their consumer appeal.

#### CONCLUSIONS

It is the belief of the authors that present-day quality in sweet cream as distributed in urban centers merits careful consideration of a number of characteristics not recognized in the score card now used interchangeably for milk and cream. At the same time, modern practice makes a different weighting of individual qualities appear desirable. A separate score card for cream is suggested, with proposed methods for judging the respective characteristics, and details of application are given. The suggested scheme has received practical trial in a number of commercial control laboratories, where it has proven workable and convenient, reflecting in an exact manner the differences which are distinguishable in a general way between samples of cream. The official adoption of such a score card, or some suitable modification of the same, would provide both the academic and the industrial investigator with a recognized standard method for evaluating cream quality as it is offered to the public under modern marketing conditions.

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# FACTORS AFFECTING THE ACTIVITY AND HEAT RESISTANCE OF SWISS CHEESE STARTER CULTURES. I. INFLUENCE OF TIME AND TEMPERATURE OF INCUBATION<sup>1</sup>

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Frazier and coworkers (7), in their studies on the bacteriology of Swiss cheese, found that cultures of *Lactobacillus helveticus* 39a, incubated at 38° to 39° C., developed more actively during the manufacture of Swiss cheese than did those incubated at 35° to 37.5° C. Unpublished results indicated that when *L. helveticus* had been grown in milk at 30° C. for 24 hours, it showed definitely less activity in the cheese on the press than when it had been grown at 37° C. for 12 hours. Despite the fact that cultures grown at the two temperatures produced about the same amount of titratable acid at the end of their respective incubation periods and presumably were of approximately the same maturity, the culture grown at the higher temperature had the greater ability to withstand heating.

During the manufacture of Swiss cheese, the temperature of the curd is raised in a period of about 30 minutes from the average setting temperature of 33° C. to an average cooking temperature of 53° C. The curd is held at or slightly below this temperature for about 30 minutes, after which it is removed from the kettle and placed in the press. The temperature falls very slowly during the next 24 hours, particularly in the center of the cheese. It is obvious then that any organisms which develop in the early hours on the press must be both heat resistant and capable of developing readily at temperatures near their maximum.

A study was made to determine the influence of time and temperature of incubation on heat resistance and activity following the heating of certain commonly used Swiss cheese starter organisms.

The literature on influence of time and temperature of incubation on heat resistance of bacteria has been reviewed briefly by Elliker (4) and by Elliker and Frazier (5).

Numerous investigations have demonstrated that age of bacterial cells markedly influences their resistance to heat. Frazier *et al.* (7) have shown the importance of age of the cultures. The temperature inducing greatest heat resistance of vegetative cells has been found to vary with the type of organism. For example, Claydon (2) and Anderson and Meanwell (1) reported that *Streptococcus lactis* and a thermophilic streptococcus, respectively, showed greatest heat resistance when grown at temperatures below the

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optimum for growth. Dorner and Thöni (3) demonstrated that there was little difference in heat resistance of cultures of *Bacterium acidi propionici* grown at 22° and at 30° C. Unpublished results obtained at the Washington laboratories of the Bureau of Dairy Industry indicated that propionic acid bacteria grown at temperatures near their optimum were more resistant to heat than those grown at lower temperatures. Results reported by Frazier and coworkers (7), and by Elliker and Frazier (5) demonstrated that with certain organisms incubation at temperatures near or above the optimum for growth might result in greater heat resistance and activity following heating than would result from incubation at a lower temperature.

#### EXPERIMENTAL

In this investigation on the heat resistance of non-spore forming, thermotolerant bacteria, *Lactobacillus helveticus* 39aW and *Streptococcus thermophilus* C-3, the organisms used by Frazier *et al.* (7), were used. In part of the work several other species of *Lactobacillus* and *Streptococcus* were employed.

The usual method of measuring heat resistance of a culture is to determine the number of organisms present both before and after heat treatment by plate counts and from these calculate the percentage surviving. This method is not adapted for the study of the heat resistance of *L. helveticus* and *Str. thermophilus* because of the tendency of these organisms to form long chains, particularly in young cultures and at high temperatures. For this reason activity following heating of the culture was followed by direct microscopic counts, and by acid titrations made before and at varying intervals following the heat treatment. This method measures the ability of the culture to grow and ferment after treatment. The main disadvantage is that it does not determine the percentage survival of cells. In these studies, a measure of the activity following heating was used more than the plate count method.

The cultures were carried in tubes of sterile reconstituted skim milk, always prepared from the same lot of skim milk powder. Unless otherwise indicated, one per cent of inoculum was used. Stock cultures were incubated at 37° C. for 16 hours after which they were kept at 10° C. until the next transfer one week later. Determination of heat resistance or of activity after heating of a culture was carried out on an inoculating culture which was prepared by transfer of one per cent of inoculum from the most recent stock or mother culture to 100 cc. of milk. Unless otherwise indicated the incubation was the same as that of the stock or mother culture. Following incubation, 0.25 per cent of culture was transferred from the inoculating culture to a liter flask containing 450 cc. of freshly steamed and cooled sterile reconstituted skim milk. The temperature of the milk in the flasks was raised in a period of about five minutes to the temperature of

heat treatment, usually 60° C., maintained for 30 minutes and then lowered to the temperature of subsequent incubation. It was believed that this treatment reasonably simulated the exposure which starter cultures might receive in the manufacture of Swiss cheese. Incubation following heat exposure was carried out in thermostatically controlled water baths. The rate of growth following the heat treatment of the cultures was determined by direct microscopic counts of living cells according to the method of Frazier and Boyer (6), and also by determinations of pH and titratable acidity. Samples were removed for these determinations before and at definite intervals following the heat treatment.

*Influence of temperature of incubation on activity following  
heat treatment*

In this experiment cultures were carried at 30°, with transfers every 24 hours, and at 35, 37, 40 and 42° C. with transfers every 12 hours. These are incubation periods commonly used in carrying starter cultures in actual practice. It was believed that growth at temperatures in this range might yield some knowledge of the effect of incubation temperature on heat resistance of the organisms, and that consequently the studies might be limited to the influence of two or three different temperatures. Such a procedure would then allow more detailed and better controlled experiments than would be possible if a greater number of temperatures were used. It was realized that the incubation temperatures and times used resulted in cultures whose maturity, as indicated by titratable acidity and pH of inoculating cultures, varied to some extent. Nevertheless, results obtained by the carefully controlled methods used should give some indication of the effect of temperature and should suggest whatever subsequent modifications of methods might be necessary.

Figures 1 and 2 show the relative activity following heat treatment of cultures carried at the various temperatures. Growth curves constructed from direct counts indicate the rate of growth at 37° C. following heat treatment. Also included are titratable acidities and pH values for the first eight hours and the 24th hour, as well as acidities of the inoculating cultures. Samples removed from the flasks of milk before heat treatment are designated in the figures as 00 hour samples; those removed immediately after heat treatment and cooling to 37° C. are designated as 0 hour samples.

The activity of the inoculating culture following heat treatment could be predicted from its titratable acidity at the time it was used. Repeatedly inoculating cultures with low titratable acidities were found to be inactive after the heat treatment. The titratable acidity produced in an inoculating culture within a given time at a given temperature, therefore, indicates how active that culture will be following subsequent heat treatment. Frazier *et al.* (7) have emphasized the importance of a certain titratable acidity in

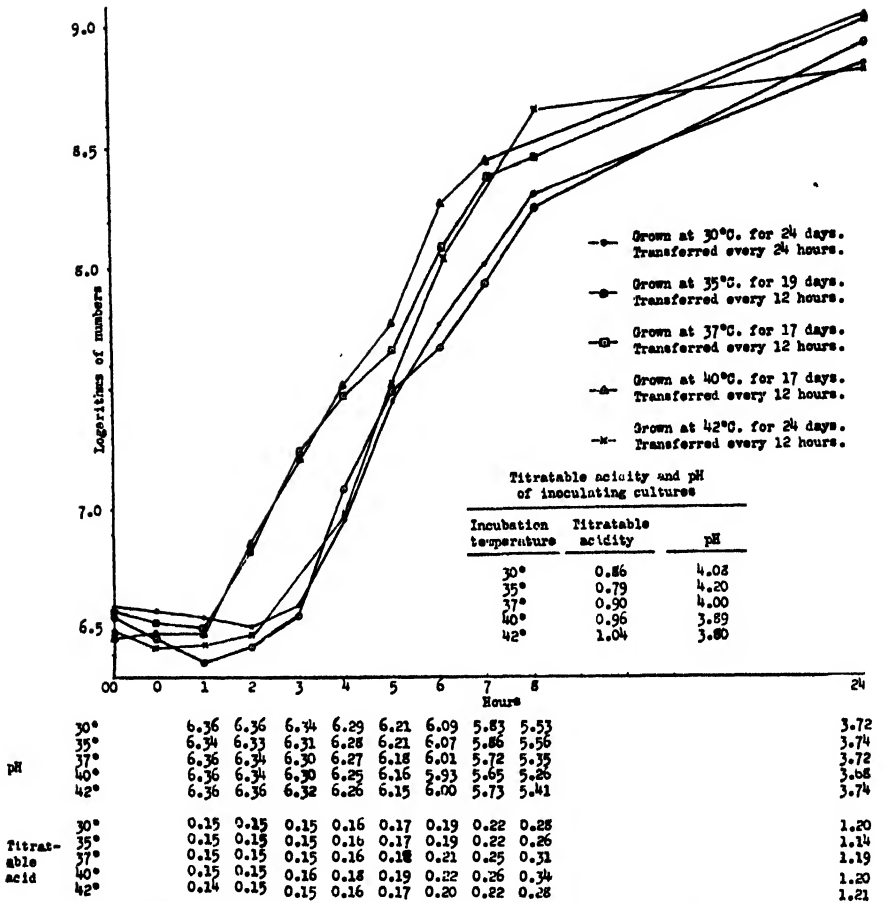


FIG. 1. Growth curves and acid production at 37° C. of cultures of *Lactobacillus helveticus* grown at different temperatures and heat shocked at 60° C. for thirty minutes.

bulk starter cultures within a given incubation time at a given incubation temperature if the starter organisms are to be active during the manufacture of Swiss cheese.

Examination of Figure 1 leaves little doubt that the 37° and 40° cultures were superior to the others. The pH and titratable acidity of the respective inoculating cultures indicate that those grown at 30° and 35° had not reached as high a stage of maturity as had the 37°, 40° and 42° C. cultures. The activity of all cultures except the 42° was directly proportional to the titratable acidities of the inoculating cultures. This indicates that the maturity of the cells in the inoculating culture may influence their subsequent heat resistance. The counts were higher in those cultures grown at the lower temperatures, therefore the original number of organisms present before heat treatment did not greatly influence the results.

TABLE 1

*Influence of incubation temperature of mother cultures on the activity of Lactobacillus helveticus and Streptococcus thermophilus at 37° C. (98.6° F.) following heat shocking*

Culture	Incubation time and temperature	Number of transfers at respective temperatures	Titrate-ble acid of inoculating culture	Time and temperature of heat shocking	Time of incubation following heat shocking	Drop in pH
			<i>Per cent</i>		<i>Hours</i>	
<i>L. helveticus</i>	12 hrs. 37°	1	0.87	30' 65°	13	.36
	12 hrs. 40°	1	0.99	30' 65°	13	.29
<i>L. helveticus</i>	12 hrs. 37°	34	0.90	30' 60°	6	.35
	12 hrs. 40°	34	0.96	30' 60°	6	.43
<i>L. helveticus</i>	12 hrs. 37°	58	0.92	30' 62.5°	8	.86
	12 hrs. 40°	58	1.11	30' 62.5°	8	.98
<i>Str. thermophilus</i>	12 hrs. 37°	1	0.63	30' 65°	5	.22
	12 hrs. 40°	1	0.67	30' 65°	5	.00
<i>Str. thermophilus</i>	12 hrs. 37°	4	0.64	30' 70°	12	.36
	12 hrs. 40°	10	0.68	30' 70°	12	.04
<i>Str. thermophilus</i>	12 hrs. 37°	16	0.64	30' 60°	5	1.00
	12 hrs. 40°	16	0.67	30' 60°	5	.86
<i>Str. thermophilus</i>	12 hrs. 37°	24	0.66	30' 65°	5	.16
	12 hrs. 40°	24	0.65	30' 65°	5	.01

Counts and pH values following heating of cultures of *Str. thermophilus*, shown in Fig. 2, indicated that the 30° and 35° cultures initiated growth first, closely followed by the 37° C. culture. The acidities of the 30° and 35°, and possibly the 37° cultures, are lower than acidities of cultures grown at the other temperatures.

The differences in rate of acid formation at 37° C. following heat treatment were slight, yet they were significant. This fact was emphasized in later experiments in which cultures were incubated at temperatures near their maximum following heat treatment.

*Effect of incubation of L. helveticus and Str. thermophilus at 37° and 40°, with transfers every 12 hours, on their activity following heat treatment*

The results shown in Figures 1 and 2 demonstrate that growth at 37° and 40° C. appeared to increase slightly the heat resistance of *L. helveticus* and that there was a slight superiority of the 40° over the 37° culture. From the results of Frazier *et al.* (7) and those obtained in this work, it seemed logical to assume that if the effect of temperature on heat resistance of

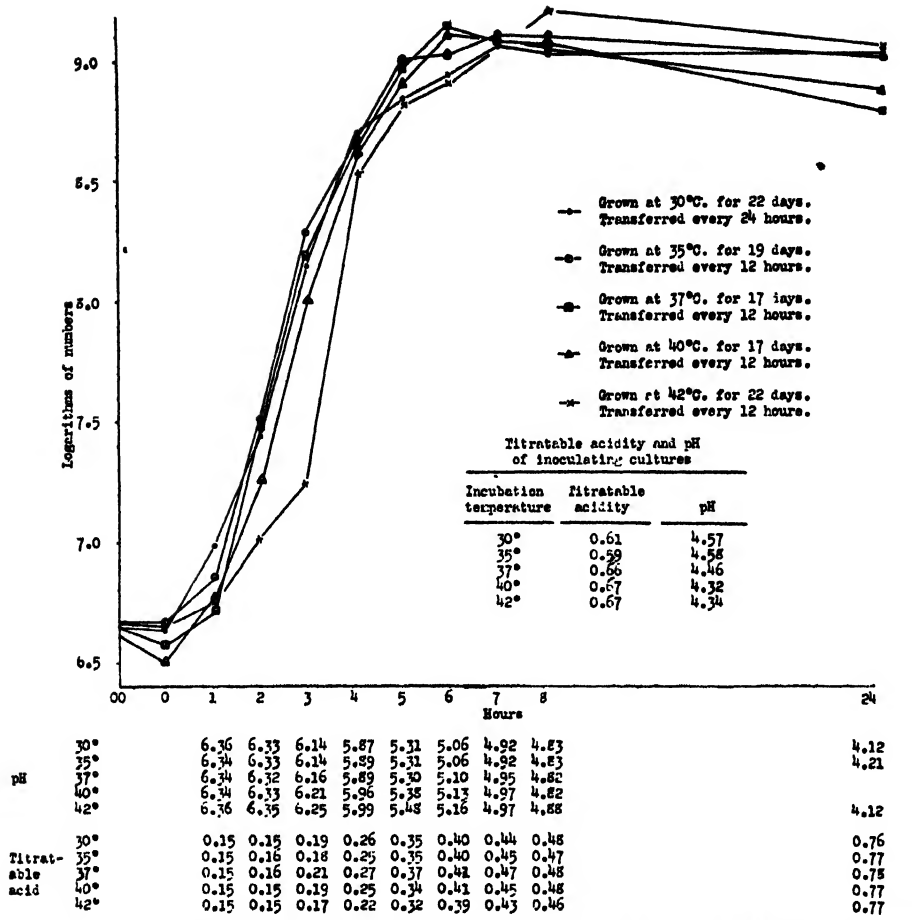


FIG. 2. Growth curves and acid production at 37° C. of cultures of *Streptococcus thermophilus* grown at different temperatures and heat shocked at 60° C. for thirty minutes.

these organisms was significant, carefully controlled, parallel studies on cultures carried at 37° and 40° should bring out differences between them. Because the higher incubation temperatures, such as 40° C. and above, apparently were unfavorable for *Str. thermophilus*, growth of this organism at 37° and 40° might result in an effect opposite to that obtained with *L. helveticus*. Accordingly, these two organisms were carried for a varying number of transfers at 37° and 40° with transfer every 12 hours. In certain cases cultures were heat shocked after only one transfer, in other cases after numerous transfers. The temperature of heat shocking was altered in certain experiments in order to emphasize differences not brought out by a less severe heat exposure. Because the presentation of all the pH values and counts would require a large number of tables, the change in pH

during a definite period following heat treatment is tabulated rather than the pH values, and counts for every individual hourly determination. It will be observed that in the tables the intervals between time of heat shocking and time of determination of acidity are varied in proportion to rate of acid formation following heat treatment.

The results shown in Table 1 indicate that following one transfer at the two temperatures, the 37° *L. helveticus* culture was better able to withstand the heat treatment, but after 34 transfers, the 40° appeared superior to the 37° culture. After 58 transfers, the 40° C. culture was still superior. It is apparent, then, that some change had taken place in the comparative resistance of the two cultures during continuous transfer at the two temperatures. *Str. thermophilus* exhibited a behavior altogether different from that of *L. helveticus*. Results in Table 1 indicate that after one transfer, the 37° *Str. thermophilus* culture was decidedly the more resistant to heat exposure. In the five hours following heat shocking the pH of the 37° culture dropped 0.22 while no measurable acid was formed in the 40° culture. After numerous transfers, similar results were obtained. Heat shocking at 65° rather than 60° emphasized this difference. After the twenty-fourth transfer, the 37° culture lowered the pH a total of 0.16 during the first five hours after heat shocking while the 40° culture lowered it only 0.01.

It was demonstrated further that the greater heat resistance of the 40° *L. helveticus* culture was not eliminated by one transfer at a temperature of 37° C. After the mother cultures had been carried at 37° and 40° C., respectively, for 60 days, one inoculating culture was inoculated from the 37° mother culture and incubated at 37° C. for 12 hours. A second inoculating culture was inoculated from the 40° mother culture and incubated at 40° for 12 hours. A third was inoculated from the 40° mother culture and incubated at 37° C. for 12 hours. The 40° culture grown continuously at 40° was more resistant than the same 40° culture grown for one transfer at 37°. However, both of these cultures were more resistant than the 37° culture grown continuously at 37° C.

*Effect of continuous transfer of mother cultures at 37° and 40° C. on their activity at temperatures near their maximum after heat shocking*

In previous experiments a temperature of 37° C. was employed following heat shocking to compare ability of cultures to develop following exposure to heat. In the manufacture of Swiss cheese any organisms which are to develop in the earlier stages (the time when cultures of *Str. thermophilus* and lactobacilli should be active) must be able to initiate growth at the high temperature of the curd at the time it is placed in the press and must continue to grow at the high temperatures which are maintained while the

cheese is on the press. Results above show definite but not pronounced differences between *L. helveticus* grown at 37° and at 40° C. Nevertheless field results and those of Frazier *et al.* (7) demonstrated that a slightly higher incubation temperature of bulk starters resulted in greater activity of organisms when the curd was on the press. It was considered possible that the apparent superiority of the 40° over the 37° C. culture might be due to the fact that the change in a culture produced by continued growth at the higher incubation temperature was the ability to grow well subsequently at higher temperatures (*e.g.*, near the maximum temperature) rather than any effect on survival and on activity of cells at 37° after heating. For this reason in this and in certain of the following experiments, after the cultures were heat shocked at 60° C. for 30 minutes, they were incubated at temperatures near their maximum. Counts and pH determinations were made at definite intervals to compare development at the higher tempera-

TABLE 2

*Influence of incubation temperature of mother cultures on activity of Lactobacillus helveticus and Streptococcus thermophilus at temperatures near their maximum following heat shocking at 60° C. for thirty minutes*

Culture	Incubation time and temperature	Number of transfers at respective temperature	Titrateable acid of inoculating culture	Temperature of incubation following heat shocking	Time of incubation following heat shocking	Drop in pH
			<i>Per cent</i>	<i>° C.</i>	<i>Hours</i>	
<i>L. helveticus</i> . . .	12 hrs. 37°	1	0.88	44	8	.60
	12 hrs. 40°	1	1.06	44	8	.35
<i>L. helveticus</i> . . . . .	12 hrs. 37°	18	0.80	44	8	.09
	12 hrs. 40°	18	1.03	44	8	.59
<i>L. helveticus</i> . . . . .	12 hrs. 37°	70	0.95	44	8	.23
	12 hrs. 40°	70	1.12	44	8	.77
<i>L. helveticus</i> (a)	12 hrs. 37°	96	0.89	44	9	.04
(b)	12 hrs. 40°	96	1.03	44	9	.45
(c)	14 hrs. 37°	96	1.00	44	9	.05
(d)	10 hrs. 40°	96	0.94	44	9	.43
<i>Str. thermophilus</i> ...	12 hrs. 37°	1	0.70	48	4	.73
	12 hrs. 40°	1	0.70	48	4	.68
<i>Str. thermophilus</i> .	12 hrs. 37°	140	0.68	48	4	.56
	12 hrs. 40°	140	0.71	48	4	.31
<i>Str. thermophilus</i> ...	12 hrs. 37°	1	0.71	48	4	.88
	12 hrs. 37°	150	0.70	48	4	.51

(a) and (c) = Inoculated from mother culture carried at 37° C. for 48 days and transferred every 12 hours. Table shows incubation time and temperature of inoculating cultures.

(b) and (d) = Inoculated from mother culture carried at 40° C. for 48 days and transferred every 12 hours. Table shows incubation time and temperature of inoculating cultures.

tures following heating. Preliminary tests indicated that the most suitable incubation temperatures for such experiments were 44° for *L. helveticus* and 48° C. for *Str. thermophilus*.

The results, shown in Table 2, demonstrate certain differences in cultures of *L. helveticus* carried at 37° and 40° which were not brought out by incubation at lower temperatures following heating. After the first transfer of the mother cultures at 37° and 40°, the 37° appeared more resistant. After 18, 70 and 96 transfers, the 40° culture was without doubt the more active at 44° after the exposure to heat. The change in activity following heating was in the same direction as in previous experiments (i.e., after numerous transfers the 40° appeared more heat resistant than the 37° culture). After the ninety-sixth transfer, the 37° mother culture was transferred to two inoculating cultures which were incubated at 37° for 12 and 14 hours, respectively. The 40° mother cultures were transferred to inoculating cultures which were incubated at 40° C. for 10 and 12 hours, respectively. The titrations of the 12 hour, 37° and the 10 hour, 40° cultures were 0.89 per cent and 0.94 per cent respectively. Those of the 14 hour, 37° and 12 hour, 40° cultures were 1.00 and 1.03 per cent. In spite of the close approximations in titratable acidities of mother cultures, the 40° culture lowered the pH after heating 0.43 and 0.45 as compared to 0.04 and 0.05 for the 37° culture. The greater maturity of the 40° inoculating cultures apparently was not responsible for their greater resistance and their activity at 44° following heating. Similar differences in heat resistance were obtained with stock cultures carried at 37° and 40°, respectively, and transferred every week.

The results obtained with *Str. thermophilus*, shown in Table 2, on the whole are similar to those obtained when an incubation temperature of 37° rather than 48° C. was employed following heat treatment.

When microscopic counts were made of cultures incubated at temperatures near their maximum following heating, irregular results were often obtained because of the formation of extremely long entangled chains and because unusually high numbers of the cells were gram-negative, due possibly to a change in staining properties after rapid growth at the high temperature.

*Influence of time and temperature of incubation on activity of L. helveticus and Str. thermophilus at temperatures near their maximum following heat treatment*

Previous experiments were concerned chiefly with the influence of incubation temperature and although certain allowance had been made for it, the fact soon became evident that greater emphasis must be placed on effect of time of incubation. The cultures of *L. helveticus* used for this experiment were being transferred daily, incubated for 14 hours at 37° or 40° and then placed at 10° for 10 hours until time for the next transfer. Those of *Str. thermophilus* were being transferred daily, incubated for 12 hours at 37° and 40° and then placed at 10° for 12 hours until time for the next

transfer. Results indicated that 40° cultures of *L. helveticus* and 37° cultures of *Str. thermophilus* were superior in each case. But as in earlier experiments, it was believed possible that an incubation time of 14 hours for *L. helveticus* and 12 hours for *Str. thermophilus* was not the optimum. Accordingly, fresh transfers of these two organisms, carried in the manner described, were used to prepare inoculating cultures which were then incubated at 37° and 40° for periods varying from 6 to 16 hours. The inoculating cultures were inoculated at such intervals that they might all be removed and heat shocked simultaneously. Titrations and pH determinations made of inoculating cultures indicated their relative maturity. Table

TABLE 3

*Influence of time and temperature of incubation on activity of Lactobacillus helveticus at 44° C. following heat shocking at 60° C. for thirty minutes*

Time and temperature of incubation of mother culture	Number of daily transfers of mother culture by this method	Time and temperature of incubation of inoculating culture	Titratable acid of inoculating culture	Drop in pH during first eight hours following heat shocking
			<i>Per cent</i>	
14 hrs. 37° 10 hrs. 10°	30	7 hrs. 37°	0.49	.00
	30	12 hrs. 37°	0.96	.29
	30	16 hrs. 37°	1.14	.26
14 hrs. 40° 10 hrs. 10°	30	7 hrs. 40°	0.64	.01
	30	12 hrs. 40°	1.10	.36
	30	16 hrs. 40°	1.26	.23
14 hrs. 37° 10 hrs. 10°	65	8 hrs. 37°	0.41	.03
	65	12 hrs. 37°	0.86	.42
	65	16 hrs. 37°	1.11	.46
14 hrs. 40° 10 hrs. 10°	65	8 hrs. 40°	0.56	.05
	65	12 hrs. 40°	1.04	.51
	65	16 hrs. 40°	1.25	.39

3 demonstrates that under the conditions employed in this experiment, the time of incubation definitely influenced the ability of *L. helveticus* to develop after heat shocking. Twelve and 16 hour cultures of *L. helveticus* were far more active following heat treatment than were the seven and eight hour cultures. As shown in Table 4, there was little measurable difference in heat resistance of cultures of *Str. thermophilus*, grown for periods of 6, 12, and 16 hours, respectively. In another trial there was little difference between 8 and 16 hour, 37° cultures of *Str. thermophilus*, and no significant difference between 8 and 16 hour, 40° cultures.

#### DISCUSSION

The results indicated that when *L. helveticus* was carried at temperatures of 30°, 35°, 37°, 40°, and 42° C. for the incubation periods commonly employed in actual practice for starter cultures of this organism and other related lactobacilli, the heat resistance of cultures incubated at temperatures of 37° and 40° was greater than that of cultures incubated above or

TABLE 4

*Influence of time and temperature of incubation on activity of Streptococcus thermophilus at 44° C. following heat shocking at 60° C. for thirty minutes*

Time and temperature of incubation of mother culture	Number of daily transfers of mother culture by this method	Time and temperature of incubation of inoculating culture	Titrateable acid of inoculating culture	Drop in pH during first eight hours following heat shocking
			<i>Per cent</i>	
12 hrs. 37° 12 hrs. 10°	29	8 hrs. 37°	0.58	1.03
	29	12 hrs. 37°	0.68	1.02
	29	16 hrs. 37°	0.77	1.02
12 hrs. 40° 12 hrs. 10°	29	8 hrs. 40°	0.62	1.03
	29	12 hrs. 40°	0.72	.76
	29	16 hrs. 40°	0.77	.92
12 hrs. 37° 12 hrs. 10°	53	6 hrs. 37°	0.50	1.06
	53	12 hrs. 37°	0.71	1.05
	53	16 hrs. 37°	0.78	1.11
12 hrs. 40° 12 hrs. 10°	53	6 hrs. 40°	0.52	1.06
	53	12 hrs. 40°	0.73	1.08
	53	16 hrs. 40°	0.79	1.05

below these temperatures. When cultures were carried at 37° and 40° and transferred every 12 hours, it was found that the first transfer of the 37° cultures was fully as resistant as and sometimes more resistant to heat than the first transfer of the 40° culture. Titrations of the inoculating cultures showed that the 40° culture was more mature than the 37° culture. As the consecutive 12 hour transfers at the two respective temperatures were continued, the 40° culture gradually became the more resistant. These results indicated, then, that the higher temperature of incubation was responsible for the increased heat resistance. The 40° was more mature at the time of every transfer than the 37° culture and any advantage in heat resistance due to greater maturity may have accumulated over a number of successive transfers. On the other hand, the 40° culture was more mature at the first transfer yet less heat resistant than the 37° culture. This fact would suggest that maturity of the cultures was not a major factor in the development of differences in heat resistance between 37° and 40° cultures after repeated transfer. The results strongly indicate, then, that the higher incubation temperature was primarily responsible for greater heat resistance. Differences in numbers of organisms in the respective cultures before heating were not great enough to cause significant differences in the numbers surviving heat treatment.

Because the greater heat resistance of the 40° culture was not lost by one transfer at 37°, it would appear that the increased heat resistance of the 40° cells may have been permanent enough to survive several generations of growth at the lower temperature.

Incubation of heat shocked cultures at temperatures near their maximum emphasized the difference between 37° and 40° cultures of *L. helveticus*.

*cus*. Results to appear in a later paper indicate that the number of active cells present in a culture largely govern its ability to develop at 44° C.

*Streptococcus thermophilus*, when carried at different temperatures by the same methods employed for *L. helveticus* demonstrated an entirely different behavior. Growth at 30°, 35° and 37° C. yielded cultures which were more active following heat treatment than were those grown at 40° and 42° C. When cultures of this organism were grown at 37° and 40° and were transferred every 12 hours, the 37° culture was more resistant than the 40°, both after one and after numerous transfers. These heat resistant studies were made on cultures which were about at the stage of maximum heat resistance. Titratable acidities of inoculating cultures indicated that possibly the greater amount of acid produced by the 40° as compared with the 37° culture had an injurious effect on the cells and therefore lowered their heat resistance. However, when cultures were incubated at 37° and 40° for periods varying from six to 16 hours, no great difference in heat resistance was obtained. This fact would minimize the influence of the greater amount of acid produced in a 12 hour incubation period at the higher temperatures on the heat resistance of *Str. thermophilus*. The results, as a whole, demonstrate that growth of *Str. thermophilus* at lower temperatures results in greater activity and heat resistance than growth at the higher temperatures used in these studies. *Str. thermophilus* has a higher maximum temperature and was apparently influenced less by the incubation time and type of milk in which it was grown than *L. helveticus*. This explains why it is easier to obtain active starter cultures of *Str. thermophilus* than of *L. helveticus*. The importance of the culture medium in developing active starter cultures will be discussed in a following paper.

The results obtained by incubation of cultures of *L. helveticus* and of *Str. thermophilus* at temperatures near their maximum following heat shocking explain why certain Swiss cheese starter cultures which show no outward differences in activity still vary so widely in their ability to develop at the high temperatures prevailing in Swiss cheese on the press. The high temperature effects a rather delicate balance in that it determines largely whether or not the starter organisms will develop at an early hour. If the culture is weak, it may not develop until the temperature falls to a favorable level. The consequent slow acid development in the curd on the press may result in serious defects in the cheese.

#### SUMMARY

1. When *L. helveticus* was carried for numerous transfers at different temperatures by methods similar to those commonly employed in handling starter cultures of this organism, those cultures carried at 37° and 40° were more active following heat treatment than were cultures carried at 30°, 35° and 42° C.

2. The 37° cultures of *L. helveticus* were more heat resistant than the 40° C. cultures after the first 12 hour transfer at the respective temperatures, but after numerous, successive 12 hour transfers at the respective temperatures, the 40° was more resistant than the 37° culture. This increased resistance of the 40° culture was not lost by one transfer at a lower temperature.

3. Cultures of *Str. thermophilus* carried for numerous successive transfers at different temperatures by the same methods used for *L. helveticus* showed greater heat resistance when grown at 30°, 35° and 37° than at 40° or 42° C. The 37° cultures were more active than were the 40° C. cultures of *Str. thermophilus* after both one and numerous successive 12 hour transfers at the respective temperatures.

4. Heat treatment in the usual manner, followed by incubation of the respective cultures at temperatures near their maximum and comparison of their subsequent rates of growth and acid production, emphasized the differences in activity following heat shocking of cultures of *L. helveticus* and *Str. thermophilus*.

5. Cultures of *L. helveticus* incubated at 37° and 40° C. for 12 to 16 hours were far more heat resistant than cultures incubated at 37° and 40° C. for seven and eight hours.

6. There was no marked difference in activity following heat treatment of cultures of *Str. thermophilus* incubated at 37° C. for periods varying from six to 16 hours, the range of incubation periods usually employed in growing starter cultures of this organism.

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#### ERRATUM

July, 1938, page 335, line 17, should read as follows: "the preparation of 0.05 N KOH and  $\text{C}_2\text{H}_5\text{ONa}$  solutions."



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## SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland	New York Association of Dairy and Milk Inspectors
International Association of Ice Cream Manufacturers	Prussian Dairy Research Institute, Kiel, Germany
International Association of Milk Dealers	State Agricultural Colleges and Experiment Stations
International Association of Milk Inspectors	
National Dairy Experiment Station, Hillerod, Denmark	The Royal Technical College, Copenhagen, Denmark
National Institute for Research in Dairying, Reading, England	United States Department of Agriculture

# ABSTRACTS OF LITERATURE ON MILK AND MILK PRODUCTS

## BACTERIOLOGY

1. **Mastitis.** K. G. WECKEL, Univ. of Wisconsin, Madison, Wisconsin. *Nat. Butter and Cheese J.* 28, 12, p. 10, June 25, 1937.

The severity of this infection of the udder determines the amount and condition of milk secreted. Differences in twenty-three properties of milk from normal and infected udders are tabulated and their possible effects on cheese are discussed. Various tests for detecting mastitis are described. Suitable precautions in applying the tests are suggested. W.V.P.

2. **Methods for Differential Staining for Dead and Living Bacteria.** EUGEN P. NADELIN, Wooster, Ohio. *Milk Dealer* 26, 10, p. 35, July, 1937.

The author reviews the literature and reports his findings on methods for differential staining for dead and living bacteria. C.J.B.

3. **Comparison of Solid with Liquid Media as a Means of Determining the Presence of Lactose Fermenting Bacteria in Pasteurized Milk.** M. W. YALE, N. Y. Agr. Exp. Sta., Geneva, N. Y. *Am. J. Pub. Health* 27, 6, 1937.

A comparison of 11 agar media showed that desoxycholate and violet red bile agar were best suited for the examination of pasteurized milk. The conditions under which it is of value to use a solid rather than a liquid medium and the significance of the red acid forming colonies which develop upon desoxycholate and violet red bile agar plates are discussed M.W.Y.

## BUTTER

4. **Paying for Milk in Whole Milk Creameries.** H. C. JACKSON, Univ. of Wisconsin, Madison, Wis. *Nat. Butter and Cheese J.* 28, 8, p. 34, April 25, 1937.

Distribution of creamery receipts on the straight-fat basis is satisfactory, even when by-products are made into dry milk, if milk tests of individual patrons do not vary widely from the plant average. When tests vary widely, an equitable method of payment is suggested which allows for differences in yield of dry milk; variations in costs of handling milk of different fat tests; and changing prices of dry milk. W.V.P.

5. **Cold Storage Lockers.** Anonymous. *Nat. Butter and Cheese J.* 28, 8, p. 6, April 25, 1937.

Many creameries and cheese factories have found a cold storage locker

system a profitable sideline. Organization, type of equipment, freezing methods and outlook for the cold storage movement are described. W.V.P.

**6. A Practical Solution to the Milk Hauler Problem.** Anonymous. Nat. Butter and Cheese J. 28, 10, p. 6, May 25, 1937.

A creamery company in Wisconsin leases trucking equipment to handle milk routes. Drivers who may be truck owners are hired to operate the trucks. Forms of contracts are shown. Reduction in costs of Workmen's Compensation Insurance and elimination of postage charges for sending checks and statements to patrons more than pay for the additional costs of Unemployment Insurance and Social Security taxes. W.V.P.

**7. Controlling the Flavor of Butter.** H. A. RUEHE, Dept. of Dairy Husb., Univ. of Ill., Urbana, Ill. Nat. Butter and Cheese J. 28, 4, p. 20, Feb. 25, 1937.

Diacyl is the cause of flavor and aroma in butter. The steam distillates from certain starters are rich in this butter flavor. Proper manipulations of starters increase the flavor yield. Distillate of standardized strength can be added to butter at salting at the rate of one part distillate to 200,000 parts of butter to produce a preferred flavor. The flavor thus produced seems to stand storage. The distillate is economical to use, but its addition to butter is illegal according to regulations of the Federal Food and Drug Administration. W.V.P.

**8. Whole Milk Movement.** Anonymous. Nat. Butter and Cheese J. 28, 4, p. 6, Feb. 25, 1937, and 28, 5, p. 6, March 10, 1937.

A survey of the Middle West indicates a general trend toward the delivery of whole milk to creameries. In Wisconsin, Minnesota and Michigan the trend is very definite; in Iowa, the Dakotas, Illinois, Kansas and Indiana the trends are noticeable. Some sources of information indicate that creameries are beginning to manufacture other types of dairy products, such as ice cream mix, evaporated milk and cheese. A map and a table are presented in the second article to show that the whole milk movement is a long time trend. W.V.P.

**9. The Butter Outlook.** H. A. Ross, Bureau of Economics, The Borden Co. Nat. Butter and Cheese J. 28, 1, p. 6, Jan. 10, 1937.

The author discusses the effect on the butter market of such factors as: number of cows; the cow cycle; geographical distribution of cows; market milk surplus; crop conditions; demand for butter; and consumer purchasing power. Production is not keeping pace with population. Consumer purchasing power is increasing. The average price of butter during the next

two or three years will probably be higher than during the last three. Ross expects decreased production now but more cows and an over-supply of milk in a few years. W.V.P.

Other abstracts of interest are numbers 14, 27, 47, and 48.

## CHEESE

### 10. Results of Treating Cheese Milk or Fresh Curds with Cured Cheese.

J. C. MARQUARDT, N. Y. State Agr. Exp. Station, Geneva, N. Y.  
Nat. Butter and Cheese J. 28, 11, p. 20, June 10, 1937.

Cured Roquefort, Limburger and Sap Sago cheese were added to milk to be used for cheddar cheese or to the cheddar curd during matting. Sap Sago produced a pleasing flavor characteristic of dried *Melilotus caerulea* when added to milk at the rate of one quarter per cent. One per cent of Roquefort or Limburger added to the milk caused little change in cheese score but five per cent added to the curd injured both body and flavor. W.V.P.

### 11. A Study of Sugaring Curds in the Manufacture of Cheddar Cheese.

J. C. MARQUARDT, N. Y. State Agr. Exp. Station, Geneva, N. Y.  
Nat. Butter and Cheese J. 28, 10, p. 14, May 25, 1937.

The addition of three quarters per cent cane sugar to ten experimental batches of cheddar cheese curd just before salting reduced the average moisture content of the cheese 1.1 per cent and increased the average score of the cheese in body 0.4 point and in flavor 0.4 point. The effect of sugar on quality may be due to decrease in moisture or to changes in curd structure, pH and bacterial influences. W.V.P.

### 12. Observations on European Cheese Production. J. C. MARQUARDT,

N. Y. Agr. Exp. Sta., Geneva, N. Y. Nat. Butter and Cheese J.  
28, 3, p. 25, Feb. 10, 1937.

Laboratories and commercial plants were visited in Germany, Holland, Ireland and England. Developments in research and plant practice are reported. Observations of quality, manufacturing process, and composition of popular kinds of European cheese are given. W.V.P.

### 13. Methods for the Manufacture of Smoked Type Cheese. J. C. MAR-

QUARDT, N. Y. Agr. Exp. Station, Geneva, N. Y. Nat. Butter and  
Cheese J. 28, 9, p. 10, May 10, 1937.

The manufacture of smoked type cheese in the United States has been limited because of the involved technique when natural smoke is used. Liquid smoke (pyroligneous acid) was used to impart smoked flavor by adding it, experimentally, at different stages of cheese making. Additions

of liquid smoke at the rate of 0.1 per cent to milk or to whey just after cutting produced a pleasant smoked flavor in cheddar and Provolona cheese.  
W.V.P.

14. **Findings in Farm Science.** Annual Report of the Director, 1935-36, Agr. Exp. Station, Univ. of Wisconsin, Madison. Bull. 438, March, 1937.

This bulletin briefly summarizes the newest findings of work just completed or in progress. The following sections are of interest to the dairy industry:—

Relation of abnormal milk to mastitis: E. G. Hastings, p. 40.

Studies of mastitis: F. B. Hadley, pp. 39, 41.

Cheese meal in pig rations: G. Bohstedt and J. M. Fargo, p. 50.

Learn how to make winter milk better: G. O. Kohler, *et al.*, p. 131.

Determine best conditions for growing Swiss cheese starter: W. C. Frazier and P. R. Elliker, p. 149.

Salting brick cheese: E. L. Byers and W. V. Price, p. 152.

Wrapper for processed cheese: H. L. Templeton and H. H. Sommer, p. 153.

Low cost milk pasteurizing equipment for cheese factories: W. V. Price and Z. D. Roundy, p. 154.

Composition of butterfat determines its stability toward oxidation: V. C. Stebnitz and H. H. Sommer, p. 156.

Research makes possible more efficient irradiation apparatus: H. H. Beck, K. G. Weckel and H. C. Jackson, p. 157.

Other abstracts of interest are numbers 46 and 48.

## CONCENTRATED AND DRY MILK

Abstracts of interest are numbers 14, 15, 16, 27, 34, 45, 47, and 48.

## FOOD VALUE

15. **Milk and Health.** A. D. BURKE, Dairy Dept., Alabama Polytechnic Inst., Auburn, Ala. Milk Dealer 26, 11, p. 31, Aug., 1937.

The author discusses the necessity, from a health standpoint, of milk in the diet.  
C.J.B.

16. **Nutritional Economics of Dietary Calcium.** FRANK L. GUNDERSON, Nutrition Lab., The Quaker Oats Co., Chicago, Ill. Am. J. Pub. Health 27, 6, p. 570, 1937.

Relative costs to the consumer of 1 gram of calcium are shown for foods relatively high in calcium content and for calcium pharmaceuticals. Milk consumption for a large proportion of the population is too low to supply

an adequate amount of calcium. The addition of calcium salts to certain foods consumed regularly by families on extremely low budgets would help significantly.  
M.W.Y.

17. **A Safeguard Against Lead Poisoning.** Anonymous. *Milk Dealer* 26, 9, p. 78, June, 1937.

A brief discussion of how milk is an important method of combating lead poisoning.  
C.J.B.

## ICE CREAM

18. **Price-Cut Protection on Package Helps Entire Sales Program.** Anonymous. *Ice Cream Trade J.* 33, 7, p. 10, July, 1937.

The "Darlene" package is the patented name of a new package of ice cream recently introduced by the Philadelphia Dairy Products Co., Philadelphia, Pa. It contains 17½ fluid ounces of ice cream and weighs 12½ to 13 ounces. French vanilla is used in combination with (1) raspberry ice, chocolate, and fresh strawberry; (2) pistachio, cherry pecan, and chocolate-marshmallows; (3) strawberry ice, caramel fudge and pineapple; (4) mint ice, chocolate, and cherry custard. The shape of the package is hexagonal and four generous portions, each containing all four flavors, can be served from it. The minimum retail price, protected by manufacturer-dealer contract, is 29 cents at present. Coupled with the promotion of "Darlene" ice cream is a merchandising program designed to help each dealer increase his business.  
W.H.M.

19. **Recent Developments in Ice Cream Research.** Anonymous. *Ice Cream Trade J.* 33, 7, p. 12, July, 1937.

This article describes the high lights of the recent meeting of the American Dairy Science Association held June 21-25 in Lincoln, Nebraska. Abstracts of papers dealing with ice cream and related subjects were presented.  
W.H.M.

20. **"Sogo" Ice Cream.** THOMAS B. HARRISON, Univ. of Tenn., Knoxville, Tenn. *Ice Cream Trade J.* 33, 7, p. 29, July, 1937.

Sogo, abbreviation for sorghum, is the name of a new flavored ice cream developed at the University of Tennessee, Knoxville, Tennessee. The flavor is produced by the addition of 1 pound of especially prepared sorghum syrup to 5 gallons of regular ice cream mix in the freezer. According to the author, a delightful flavor somewhat like caramel and butterscotch is produced.  
W.H.M.

21. **Merchandising for Greater Profits.** FREDERICK A. VAN FLEET. Anonymous. *Ice Cream Trade J.* 33, 7, p. 30, July, 1937.

The Coulter Drug Store in Lakewood, Ohio, increased its ice cream sales from 2,790 gallons in 1933 to 13,500 in 1934, 14,000 in 1935, and 14,500 in 1936. This increase was accomplished by rearranging the store, and installation of new ice cream equipment. The store specialized in high grade, store filled packages, and increased the number of flavors sold from 4 to 24. Ice Cream sales increased from 5 to 15 per cent of the total sales in the store and general business in the store also increased about 15 per cent.

W.H.M.

- 22. Routing Orders to Customers.** E. T. FOXENBERGH, Bloomer Bros. Co., Newark, N. Y. *Ice Cream Trade J.* 33, 7, p. 34, July, 1937.

The centralization of traffic functions and placing them in charge of a traffic manager has made it possible to give better and more economical service to the customers of this concern. Savings were effected by the establishment of commodity rates, new classification ratings, transit privileges and combining less-than-carload shipments out to carload shipments.

W.H.M.

- 23. Preventing Flavor Defects in Ice Cream Caused by Fat Oxidation.** C. D. DAHLE AND D. V. JOSEPHSON, Penn. State College, State College, Penn. *Ice Cream Rev.* 20, 11, p. 31, June, 1937.

When copper was introduced into the ice cream mix at the rate of two p.p.m. to accelerate the development of oxidized flavor it was found that the addition of 0.5 to 0.7 per cent of oat flour of a fine mesh delayed or prevented the onset of the oxidized flavor for several weeks. When oat flour was added to fresh cream before being frozen for storage, a definite protection was afforded the ice cream made from the cream. The use of oat flour increased the body score of the ice cream.

J.H.E.

- 24. How to Produce Quality Ice and Sherbets.** N. E. FABRICUS, Iowa State College, Ames, Iowa. *Ice Cream Rev.* 20, 9, April, 1937.

Since the flavoring material used in an ice or sherbet is comparatively more important than the flavoring materials used in ice cream, its quality should be more carefully considered. In many cases there is a tendency to add too much flavor to sherbets, and in many cases fortified flavors are used. In a set of samples exhibited at the Iowa Convention the following flavor criticisms were most common: unnatural, acid, peel oil flavor, turpentine flavor, stale milk products.

Common defects, such as softening at ordinary cabinet temperatures, a fluffy body, and settling out of the concentrated syrups, are due in part to inadequate overrun control. This can best be overcome by freezing half batches.

J.H.E.

- 25. Sodium Alginate as a Stabilizer for Ice Cream.** E. O. ANDERSON, L. R. DOWD, AND H. HELMBOLDT, Conn. State College, Storrs, Conn. *Ice Cream Rev.*, 20, 11, p. 88, June, 1937.

Sodium Alginate is as effective as gelatin as a stabilizer for ice cream.  
J.H.E.

- 26. Ice Cream Plant Layouts and Design.** C. F. WEINREICH, Cherry-Burrell Corp., Chicago, Ill. *Ice Cream Rev.* 20, 10, p. 56, May, 1937.

Detailed plans, including arrangement of equipment, are given for an ice cream plant manufacturing approximately 300,000 gallons annually.  
J.H.E.

- 27. Air Conditioning in the Ice Cream Plant.** G. O. WEDDELL, York Ice Machinery Corp., Pittsburgh, Pa. Branch. *Ice Cream Rev.* 20, 11, p. 36, June, 1937.

A year around air conditioning system cools and dehumidifies the air in summer, heats and humidifies the air in winter, cleans and circulates the air, and continuously adds to the system the desired amount of outside air brought in for ventilation. Various sections of the ice cream plant can be air conditioned to advantage.  
J.H.E.

Other abstracts of interest are numbers 2, 3, 5, 7, 9, 14, 15, 16, 33, 34, 45, 47, and 48.

## MILK

- 28. The Relationship of Composition to Quality in Milk. II.** J. C. MARQUARDT, N. Y. Agr. Exp. Station, Geneva, N. Y. *The Goat World* 21, 7, Nov., 1936.

This study was made on samples of goat's milk collected throughout the United States in the second National Goat's Milk Scoring Contest. It is probable that the results apply to other milk.

As previously established fat, total solids, reaction, and curd tension did not affect the milk flavor scores. High lactose and low salt percentages were associated with high scores for flavor of goat's milk.

The reliability of determining the central tendencies using analyses at a given place over a long period as compared to gathering analyses over a scattered area for a brief period are discussed.  
J.C.M.

- 29. Some Economic Aspects of a Milk Quality Program.** V. C. MANHART, Dept. of Dairy Husbandry, Purdue Univ., Agr. Exp. Station. *Milk Dealer* 26, 9, p. 70, June, 1937.

Recognizing that quality control must, as a matter of necessity, vary with circumstances, the Dairy Department of Purdue University has recommended as a milk quality control program for general use in Indiana that (1) all milk be graded at the plant by the senses of taste and smell; (2) that milk objectionable in flavor or high in acid content should be rejected or paid for at a lower price; (3) that milk satisfactory on the basis of flavor should be subjected to the methylene blue and sediment tests at least twice a month—with flavor, of course being determined daily.

Testing this plan in Indiana it was found to result (1) in the milk plant receiving a better quality milk which sold at one cent a quart more than the prevailing pasteurized milk price in the market; (2) bacteria counts in pasteurized milk were reduced to less than 10,000 per cc.; (3) producers received 17 per cent more than the prevailing price paid in the territory.

C.J.B.

**30. Homogenized Milk.** Milk Dealer 26, 9, p. 38, June, 1937.

A description of how homogenized milk was introduced to, and accepted by, the consumers of Elgin, Ill.

C.J.B.

**31. When Your Bottle Caps Become "The Talk of the Town."** Milk Dealer 26, 9, p. 43, June, 1937.

A description of how the Kennedy-Mansfield dairy of Madison, Wisconsin, uses the milk-bottle cap for advertising.

C.J.B.

**32. A Dentist Looks at the Milk Business.** BION R. EAST. Milk Dealer 26, 9, p. 44, June, 1937.

The author enumerates the advantages of pasteurized milk and points out the reason for the existence of the raw milk fallacy.

C.J.B.

**33. Important Sources of Micro-Organisms in Milk and Other Dairy Products.** H. H. WEISER, Dept. of Bact., Ohio State Univ. Milk Dealer 26, 9, p. 46, June, 1937.

A brief discussion is given of the principal sources of micro-organisms in milk.

C.J.B.

**34. Simplifying the Cleaning Job in the Milk Plant.** Anonymous. Milk Dealer 26, 9, p. 50, June, 1937.

Description of a new device that utilizes steam to pick up and apply liquid sterilizers and cleaning solutions in the form of highly atomized and heated spray that can be controlled as to heat, pressure, and degree of moisture.

C.J.B.

**35. Sour Cream.** Anonymous. *Milk Dealer* 26, 9, p. 60, June, 1937.

This gives the information obtained from a questionnaire to 150 leading dairies of the United States by the Owens-Illinois Glass Co. In addition to giving information as to type of container, names suggested other than sour cream, and demand, the questionnaire showed that 96 per cent of the dairies sell sour cream and 81 per cent sell it under that specific name. C.J.B.

**36. This Orange Drink Business.** Anonymous. *Milk Dealer* 26, 9, p. 64, June, 1937.

A discussion of the present status of the orange drink business based on report from milk dealers in various sections of the country concerning antagonistic legislation, period of heaviest sales, best sales outlets, and the like. C.J.B.

**37. Current Trends in Milk Consumption. . . . January-March, 1937.**

EDWARD FISHER BROWN, Milk Research Council, Inc., New York, N. Y. *Milk Dealer* 26, 9, p. 80, June, 1937.

The author gives a comprehensive report of the performance of the fluid-milk market in metropolitan New York, Boston, and Philadelphia during the period January-March, 1937. C.J.B.

**38. Oxidized Flavor in Milk.** L. M. THURSTON, Univ. of Florida. *Milk Dealer* 26, 9, p. 112, June, 1937.

The author discusses the oxidized flavor in milk as related to or affected by: (1) Contamination of milk with metals, (2) Micro-organisms and reducing agents, (3) Feed of the cow, (4) Effect of various processes, (5) Effect of sunlight. C.J.B.

**39. Refrigerated Milk Delivery.** Anonymous. *Milk Dealer* 26, 10, p. 40, July, 1937.

A description of the insulated box units, that maintain nine cases of milk at 45° F. for 24 hours with 50 pounds of ice, as used on the retail delivery trucks of the Franklin Co-Operative Creamery Association of Minneapolis. C.J.B.

**40. Dairy Plant Problems.** MILTON W. LIGHTCAP, Maintenance Service Dept., Pittsburgh Plate Glass Co. *Milk Dealer* 26, 10, p. 44, July, 1937.

The author briefly discusses moisture conditions, amount of light in the building, kind of building material used, type of construction involved, presence of acid in milk, necessity for daily washing, and other factors to consider before selecting a paint for a dairy plant. C.J.B.

- 41. Computation of Milk Products.** HANS EDEL, Gehl's Guernsey Farm, Milwaukee, Wis. *Milk Dealer* 26, 11, p. 24, Aug., 1937.

The author presents a method of standardizing milk products by the use of a chart. C.J.B.

- 42. One Cent Bottle Charge Solves Bottle Loss Problem for Sidney, Ohio, Dealers.** Anonymous. *Milk Dealer* 26, 10, p. 102, July, 1937.

An account of how (after failing to secure satisfactory results with a five-cent bottle charge) six of the eight milk dealers in Sidney, Ohio, organized as the Sidney Milk Bottle Association, introduced a one-cent bottle charge this year with the result that their bottle loss is only about one-third as great as it was a year ago. C.J.B.

- 43. Visiting a Pasteurized Milk Plant in Kiev, Russia.** Anonymous. *Milk Dealer* 26, 10, p. 54, July, 1937.

A description of a Russian pasteurized milk plant. Some of the conditions existing in Russia are also briefly described. C.J.B.

- 44. Inaugurating Grade A Pasteurized Milk in the City of Chicago.** HERMAN N. BUNDESEN, President, Board of Health, Chicago, Ill., *Am. J. Public Health* 27, 7, p. 260, 1937.

The formulation and enforcement of the standard milk ordinance of the U. S. Public Health Service adopted by the City of Chicago in January, 1935, are discussed. The sale of milk and milk products, including the ingredients of ice cream, was limited in Chicago to Grade A pasteurized and certified milk and milk products. The author feels that there has been a decided improvement in the milk supply since the ordinance was adopted and that producers, distributors and consumers are satisfied with the results. M.W.Y.

- 45. Sterilization by Radiation.** A. R. DENNINGTON, Westinghouse Lamp Co., Bloomfield, N. J. *Ice Cream Rev.* 20, 12, p. 31, July, 1937.

The author states that radiation of milk on an experimental basis has shown that about 98 per cent sterility can be obtained by treating the milk in a thin film for 40 seconds and this treatment did not change the taste. For a given expenditure of radiant energy the most effective radiation is in the region of 2,537 Ångstroms and down to 2,000 Ångstroms or even less. J.H.E.

- 46. Plastic Cream and Its Uses.** L. P. SHARPLES, Milk Processes, Inc., Philadelphia, Pa. *Milk Dealer* 26, 11, p. 44, Aug., 1937.

A description of plastic cream, how it is made, and its uses. The author gives the following uses for plastic cream: A cheap means of transporting cream, A cheap means of storing cream, For making ice cream, For making cream cheese without bag draining, For making cheese by the standard methods, For making fluid cream, For making flavored spreads, For making butter. C.J.B.

Other abstracts of interest are numbers 1, 2, 3, 6, 8, 14, 15, 16, 17, 27, 46, 47, and 48.

### MISCELLANEOUS

**47. Accident Prevention Program.** Milk Dealer 26, 11, p. 35, Aug., 1937.

A description of the safety program of Metzger Dairies of Dallas, Texas. This program uses both a bonus and penalty plan. C.J.B.

**48. Legal Problems for the Plant Manager.** LEO T. PARKER, Attorney at Law, Cincinnati, Ohio. Ice Cream Trade J. 33, 7, p. 37, July, 1937.

This article deals with the legal problems which confront owners and managers of ice cream plants. Proper conducts of business will make it possible to avoid any legal controversies. W.H.M.



# JOURNAL OF DAIRY SCIENCE

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## ABSTRACTS OF LITERATURE ON MILK AND MILK PRODUCTS

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## SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland	New York Association of Dairy and Milk Inspectors
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International Association of Milk Dealers	State Agricultural Colleges and Experiment Stations
International Association of Milk Inspectors	The Royal Technical College, Copenhagen, Denmark
National Dairy Experiment Station, Hillerod, Denmark	United States Department of Agriculture
National Institute for Research in Dairying, Reading, England	

# ABSTRACTS OF LITERATURE ON MILK AND MILK PRODUCTS

## BUTTER

- 49. Researches on the Identification of Cocoa Fat (Cocoa Butter or Cocoline) in Butter.** L. HOTOX. *Le Lait* 17, 161, p. 19, Jan. 1937.

None of the various fat indices in themselves are satisfactory for determining the adulteration of butter with 10 per cent or less cocoa butter as these indices in butter alone show sufficient variation to conceal the effects of the addition of 10 per cent cocoa butter. By combining several of the indices a value may be obtained by which 10 per cent of added cocoa butter can be detected. Thus the index of volatile soluble acids in butter is 30 and that of the volatile insoluble acids is 2.5 and the refractive index of the latter is 20. If 10 per cent of cocoa butter is added to the butter, these values theoretically become 28, 3.5 and 17 respectively. For butter the product of the index of the soluble volatile acids and the refractive index divided by the index of volatile insoluble acids is 24 while, when the butter contains 10 per cent of added cocoa butter, the value becomes 13.6. It is believed that a value of 20 or above calculated as given may be taken to indicate pure butter fat. As an indication of the presence of cocoa butter the value should be 15 or lower. Values between 15 and 20 are doubtful cases. Twenty of such doubtful cases were found on the analysis of 156 samples. The method thus allows the detection of adulteration with 10 per cent of cocoa butter in a majority of cases.

A.H.J.

## CHEESE

- 50. Reasons for Authorizing Sudan IV for the Coloration of Cheese Surfaces.** JEAN PIEN, Paris, France. *Le Lait* 17, 162, p. 123, Feb. 1937.

Alkanet is the only fat-soluble dye that is an authorized food color in France. This dye is not satisfactory when used for coloring cheese rinds. It is variable in color and has different colors at different H ion concentration. Moreover, when dissolved in paraffin and the paraffin heated to 130° C. to 150° C. (266° F. to 302° F.) which is the desirable temperature for applying the paraffin to the cheese, the dye is discolored. Sudan IV is not subject to these disadvantages. When fed to rats at the rate of 1 milligram per day Sudan IV was shown to have no ill effects. Also the cheese rind is not consumed but is cut from the cheese and discarded. The Dutch have been using Sudan IV to color their cheeses for a long time and no ill

effects have been reported. It is recommended that Sudan IV be approved as a dye for the paraffin that is applied to cheese surfaces. A.H.J.

**51. The Salting and Cooking of Curds in the Manufacture of Several Varieties of Cheeses.** J. C. MARQUARDT, N. Y. Agr. Exp. Sta. Geneva, N. Y. Bull. 670, July, 1936.

A study has been completed associating the composition and quality of five varieties of cheese with variations in salting and cooking methods.

The study revealed that cheese curds should be salted at a rate based upon the milk fat content of the milk used.

Cook variation studies added fundamental knowledge useful for further investigations dealing with cheese improvement.

The study indicated that comparable milks made into cheese produced quality cheeses upon the basis of the cheese variety made, with cheddar first followed in order by granular, Monterey, and brick.

The study indicated that quality and uniformity could not be regularly expected when making cheese by the Camosum method from the type of milk generally available. J.C.M.

**52. Methods of Making Cheddar Cheese from Milk with a Low Curd Tension Due to Latent Mastitis.** J. C. MARQUARDT AND G. J. HUCKER, N. Y. Agr. Exp. Sta., Geneva, N. Y. Tech. Bull. 242, April, 1937.

It is generally agreed that a high quality cheddar cheese cannot be made from milk produced by cows with an active mastitis infection. However, since the udder tissues of practically all cattle harbor the streptococcus associated with the common type of bovine mastitis, the infection exists either in a latent or a chronic condition in almost all producing herds.

This investigation has shown that even where the milk contains demonstrable numbers of mastitis streptococci and more than 500,000 leucocytes per cc., the milk can be made into satisfactory cheddar cheese though it may lack in normal curd-formation properties. This was accomplished by the addition of 1½ to 3 per cent of starter, or by the addition of 30 per cent hydrochloric acid at the rate of 100 cc. per 1,000 pounds of milk with a smaller amount of starter.

The study has revealed the necessity and importance of using a test like the Marshall cup test in making cheese from milk whose curd tension varies from normal. It has been established that after 9 months of curing, *Streptococcus agalactiae* were present in the cheeses made from the experimental milk with a low curd tension.

Unless even latent or chronic conditions of mastitis are eliminated from cheese milk herds or the milk is especially handled during the making, losses may be experienced. J.C.M.

## CHEMISTRY

- 53. The Identification of Neutralized Milk.** M. R. BAETSLE. Dir. of the Lab. for Chemistry and Bacteriology of the City of Gand. *Le Lait* 17, 162, p. 141, Feb., 1937.

Various methods of determining whether sodium bicarbonate or sodium carbonate have been added to milk in order to neutralize lactic acid are reviewed. These methods are in general unsatisfactory or of doubtful value. Since the sodium content of milk is relatively constant the investigator recommends its determination in order to ascertain neutralization and the extent of such neutralization. The sodium to phosphate ratio may also be of value in determining neutralization. Various methods of determining sodium are discussed. Since all such methods require much time a short method for detecting neutralized milk is desired. Such a method consists in adding 30 cc. of methyl alcohol to 20 cc. of milk. If the milk has not been neutralized, rapid coagulation will take place and clear serum will form. If the milk has been neutralized, coagulation will not take place and the serum will be turbid. Moreover the serum from neutralized milk will have a pII value of 6.5 or above sometimes as high as 8.5. Very fresh milk may not curdle rapidly to yield curd and clear serum but the pH value of the serum will serve to indicate whether or not it has been neutralized. A.H.J.

- 54. The Ammonia Content of Milk and Its Determination.** S. NIEMCZYCKI AND K. GERHARDT, Inst. of General Chemistry and of Dairy Science of the Academy of Veterinary Medicine at Lwow, Poland. *Le Lait* 16, 160, p. 1050, Dec., 1936.

The ammonia was removed from milk by distillation under vacuo at 40° C. (104° F.). The ammonia in the distillate was determined by Nesslerization. In 43 samples of fresh milk the quantity of ammonia varied between 0 and 2.18 milligrams per liter, the average being 0.75 milligram per liter. The ammonia content of milk from the individual quarters of the same cow at the same milking showed appreciable variation just as in the milk of different cows. The heating of milk above 45° C. (113° F.) results in increasing the ammonia content of the milk due to decomposition of the protein. The ammonia contents of pasteurized, sterilized, and condensed milks are being determined. A.H.J.

- 55. Casein as a Primary Material for the Chemical Industry.** G. GENIN. Paris, France. *Le Lait* 16, 160, p. 1083, Dec., 1936.

The chemical composition of rennet casein and of acid and natural sour casein is given. Differences in chemical properties of the various types of casein are described. Various uses of casein in industry are described and the properties of the caseins for specific uses are discussed. A.H.J.

**56. The Phosphatide Content of Milk.** BRUNO REWALD. *Le Lait* 17, 163, p. 225, March, 1937.

The methods that have been used for determining the phosphatide content of milk have yielded variable results. A new method is therefore proposed. Whole milk powder is first prepared from milk. A kilogram of this powder is subjected to 8 extractions with cold acetone in order to remove the true fatty material. (220 grams of fatty material were obtained. This showed only minimal traces of phosphorus.) After evaporation of the acetone, the fat-free powder was subjected to an extraction with a mixture of methyl alcohol and ethyl alcohol, first cold then hot. This operation was repeated 5 times. However, as phosphatides were not completely removed, 3 other extractions were made with a mixture of 80 parts of ethyl alcohol and 20 parts of benzol. Additional quantities of phosphatides were thereby extracted. At the same time considerable quantities of sugar were extracted. The extracts were combined, cooled and then filtered and the residue washed several times with the alcohol-benzol mixture and finally with pure benzol. The alcohol and benzol were removed by evaporation and the residue dried under vacuo and then taken up in benzol. An oily mass was thereby obtained indicating that considerable fat had remained in the milk powder to be subsequently extracted in the phosphatide extractions. The fat was extracted and the phosphatides precipitated by the addition of acetone. The precipitate was filtered off, then dissolved in benzol and a precipitate again obtained by the addition of acetone. The total phosphatides obtained on repeated purifications with acetone was 2.4 grams of which the phosphorus content was 3.92 per cent. In the mixture of phosphatides 58.3 per cent were soluble in ethyl alcohol and 41.7 per cent were insoluble in alcohol. The phosphorus content of the alcohol soluble fraction (lecithin) was 3.61 per cent and of the alcohol insoluble fraction (kephalin) was 3.9 per cent. Neither the lecithin nor the kephalin are pure products but mixtures of similar substances. These researches show that the phosphatide content of dry whole milk was 0.24 per cent. If the total solids content of fluid milk is 87 per cent, the phosphatide content of fresh milk would be 0.0314 per cent. The content of lecithin soluble in alcohol would be 0.0131 per cent.

A.H.J.

**57. A Study of the Fatty Matter in Goat's Milk. Its Eventual Application to the Investigation of Mixtures of Goat's and Cow's Milk.** ANDRE CHOLLET AND ANDRE CAMUS. Contribution from the Professional School of Dairy Surgeons. *Le Lait* 17, 162, p. 135, Feb., 1937.

The Crismer index is lower and the index of saponification is higher in butter from goat's milk than in butter from cow's milk although higher saponification indices are sometimes encountered in butterfat from cow's

milk. The Planchon index is notably lower in fat from goat's milk but there were wide variations of this index in the several samples investigated. The iodine number of the fat from goat's milk is in general higher than in the fat of cow's milk but the values are within the range encountered in fat from cow's milk. The variations in the Anagat and Jean oleorefractometer values are the same for the fat from both cow's and goat's milk. The soluble volatile acids are lower and the insoluble volatile acids are higher in goat's milk fat than in cow's milk fat. The ratio of the insoluble volatile acids to the soluble volatile acids in the fat is suggested as a means of detecting mixtures of cow's and goat's milk. Thus in fat from cow's milk this ratio varies from 8 to 15 while in goat's milk the variation in ratio is from 36.5 to 43.1. A.H.J.

**58. A Rapid Method for Determining Total Nitrogenous Matter in Milk for Use in Milk Control.** A. M. LEROY, Chief of Zootechnical work at the National Inst. of Agronomy. *Le Lait* 17, 163, p. 230, March, 1937.

To 10 cubic centimeters of neutralized milk in a test-tube are added 1 cubic centimeter of neutral formaldehyde and then 10 cubic centimeters of a standard alkali solution. The pink color of the mixture in the test-tube is then compared with color standards calibrated in terms of milligrams of nitrogen per liter of milk. In 35 per cent of the samples tested the agreement between this colorimetric method and the micro Kjeldahl method was within  $\pm 0.5$  milligram of nitrogen, in 32 per cent of the tests the variation was 1.0 milligram of nitrogen per liter. The variation was greater than 2.5 milligrams of nitrogen per liter in 5 per cent of the tests. A.H.J.

## CONCENTRATED AND DRY MILK

Abstracts of interest are numbers 53, 54, 56, 59, 60, 67, 71, and 72.

## FOOD VALUE

**59. Vitamins and Sterilization Under Vacuo.** JEAN PIEN, Paris, France. *Le Lait* 17, 161, p. 27, Jan., 1937.

There are two groups of methods used in preserving foodstuffs, methods which prevent the proliferation of bacteria actually present and methods which destroy the micro-organisms present and prevent subsequent contamination. The first group of methods includes preserving by cold, by drying and by the use of sugar, salt, etc. The second method involves the use of heat to sterilize the product. The effects of sufficient heat to allow sterilization of food products are discussed. Vitamins B<sub>2</sub>, D and E are not materially reduced in potency even in the presence of oxygen. Vitamins A, B<sub>1</sub>, and C are not affected to any marked extent by heating if care is taken

to prevent oxidation. A group of rats was divided into two groups, one being fed on fresh materials, the other on materials sterilized in the absence of oxygen. At the end of 5 months, the rats on fresh foods weighed 288 grams on the average while those on sterile food averaged 277 grams. The lower weight of the rats fed on the sterile diet is not thought to be due to poorer food value but to the fact that the rats ate less of it due probably to it not being so palatable as a result of the sterilization treatment.

A.H.J.

**60. The Increase in the Antirachitic Properties of Milk by Irradiation.**

G. GENIN, Paris France. *Le Lait* 17, 161, p. 47, Jan., 1937.

Accompanying the increase in antirachitic power of milk as a result of irradiation is an off-flavor of the milk. Light of wave lengths between 3100 and 2600 Angstrom units was found to be less effective in causing the development of the off-flavor in milk than light of wave length below 2600 Angstrom units.

A.H.J.

## ICE CREAM

**61. Outline of Activities of the International Association of Ice Cream**

**Manufacturers for the Year 1937.** ROBERT C. HIBBEN, Int. Assn. of Ice Cream Mfrs., Washington, D. C. November, 1937.

The principal activities of the International Association of Ice Cream Manufacturers for the year 1937 are outlined in this bulletin. A report of the activities of the affiliated organization, The Ice Cream Merchandising Institute, is also included.

M.J.M.

**62. Ice Cream Sales Index.** Statistical and Accounting Bureau, Int. Assn. of Ice Cream Mfrs., 1105 Barr Bldg., Washington, D. C. November, 1937.

This bulletin contains an analysis of ice cream sales in 1937 compared with 1936 for the United States and Canada. The survey covers the sales for the first eight months of the year. During this period of eight months, sales in 1937 for the United States were 9.15 per cent greater than in the same months of 1936. For Canada, the increase was 14.83 per cent.

M.J.M.

**63. Selling Ice Cream via Refrigerated Dispensing Machines.** Anonymous. *Ice Cream Rev.* 20, 12, p. 28, July, 1937.

Ice cream can be dispensed successfully through dry ice refrigerated dispensing machines. Two machines operated in the New York subway during three winter months averaged 140 five cent packages per machine per day. Cost of refrigeration averaged 20 cents a day. The machine

described in the article was perfected in Holland and handles chocolate covered ice cream bars. J.H.E.

- 64. Plastic Cream.** L. P. SHARPLES, Milk Processes Inc., Philadelphia, Pa. Ice Cream Rev. 21, 1, p. 31, Aug., 1937.

Plastic cream is cream of 80 to 83 per cent butterfat content with the fat in the dispersed phase of the emulsion, as contrasted with butter which has the fat in the continuous phase of the emulsion. To make plastic cream, fluid cream of any given fat content is heated, and then passed through a special centrifugal separator. It is then pasteurized and passed over a special stainless steel drum cooler. Plastic cream has many uses which include a cheap means of transporting cream, a cheap means of storing cream, for making ice cream, for making cream cheese, for making flavored spreads. It has proven especially adaptable as a fat source for ice cream.

J.H.E.

- 65. Plant Paint Problems.** MILTON W. LIGHTCAP, Pittsburgh Plate Glass Co., Pittsburgh, Pa. Ice Cream Rev. 21, 1, p. 46, Aug., 1937.

Some of the special paint problems facing the ice cream manufacturer and suggestions for solving them are given. J.H.E.

Other abstracts of interest are numbers 49, 53, 54, and 56.

## MILK

- 66. The Maintenance and Cleaning of Milking Machines.** G. GENIN, Paris, France. Le Lait 17, 162, p. 147, Feb., 1937.

The use of milking machines is not very extensive in France due to former difficulties experienced with the machine, particularly with the rubber parts. Satisfactory rubber parts have now been developed so that they can be cleaned and sterilized without being damaged. Cleaning and sterilizing with hot water, steam and hot alkaline solutions are recommended. Sterilizing with chemical disinfectants is not as satisfactory as sterilizing by other methods as the disinfectants, particularly chlorine compounds, are corrosive and reduce considerably the serviceable period of the rubber parts.

A.H.J.

- 67. Mastitis. IV. The Composition of Milk as Affected by Latent Mastitis.** A. C. DAHLBERG, J. J. KUCERA, J. C. HENING, AND G. J. HUCKER. N. Y. Agr. Exp. Station, Geneva, N. Y. Tech. Bul. 239, Nov., 1936.

Earlier investigations indicate that the chemical composition of abnormal mastitis milk is quite different from that of milk normal in appearance. More recent work indicates that a latent mastitis infection may be present

in the udder without causing any visible change in the milk. The present investigation was undertaken to determine whether there is a demonstrable relationship between the degree of infection and the chemical composition of milk normal in appearance.

Only cows having udders free from active inflammation and whose milk was normal in appearance were selected for study. These cows were divided into three groups, *viz.*, (A) no demonstrable infection, (B) slight infection and (C) pronounced latent infection but milk normal in appearance.

Composite milk samples of complete milkings from each of these groups were submitted to detailed chemical analysis. During the course of the study samples of fore-milk from each quarter of each cow were studied by bacteriological tests to determine the amount of infection present.

Only slight differences in the chemical composition of the mixed milk of these groups were found, regardless of the degree of infection, as long as the milk remained normal in macroscopic appearance. The slight differences in chemical composition included a decrease in lactose, specific gravity, skim-milk solids and curd tension while the chlorides and albumins were slightly increased. These changes in composition were not greater than variations in chemical composition of milk between two herds of the same breed.

It is concluded that milk normal in appearance is essentially normal in chemical composition. If no milk is included from inflamed, congested or injured quarters, the chemical composition of the milk from a herd will be normal in chemical composition.

It follows that earlier investigations on the chemical composition of normal milk were not affected by the possible presence of latent mastitis.

A.C.D.

#### 68. Milk. *Lancet* 233, 327, Aug. 7, 1937.

In the British Government's recently announced policy for milk, bonuses are to be paid by the Exchequer for quality milk, including accredited milk at 1½d. a gallon, and tuberculin-tested milk at 1d. a gallon for the next three years.

Of most direct concern to public health authorities and the medical profession, however, is the Government's intention that any local authority shall be able to apply to the Minister of Health or the Secretary of State for Scotland for an order requiring efficient pasteurization of milk retailed in its area, although two years would lapse before the order would take effect, and milk from tuberculin-tested herds and sterilized milk would be exempt, and milk retailed from dairies supplied by a single herd would be exempt for three years.

If advantage is taken of these powers, says the editorial, Great Britain will have gone far to put the milk in its proper place as a food, and the medical profession will confidently recommend its wider consumption.

J.A.T.

- 69. Vacuum Milk Cooler.** JOHN E. NICHOLAS, Dept. of Agr. Eng., The Penn. State College. *Refrig. Eng.* 31, 6, p. 367, June, 1936.

This method of cooling is obtained by drawing a high vacuum on the milk container. Thus cooling is done by evaporation of some of the water in milk, or approximately three pints from every 10-gallon can, when cooled through a temperature range of nearly 40 degrees. This method of cooling is more expensive than the direct immersion method, and is slower, but has the advantage of cooling the entire contents uniformly. W.D.S.

- 70. The Supply and Utilization of Milk in Pennsylvania.** T. K. COWDEN AND E. G. FOUSE. Penn. Agr. Exp. Sta., The Penn. State College Bull. 327, April, 1936.

During April, 1934, the milk dealers operating in Pennsylvania handled 323,000,000 pounds of milk and cream. Of this amount, 83 per cent was purchased as whole milk from producers. Sales of milk for fluid use amounted to 58 per cent of the total handled, for fluid cream 12 per cent, and that used in manufacturing 30 per cent. Farmers' organizations controlled 40 per cent of the total volume handled by all dealers. Shipments of milk and cream to dealers in other states amounted to 71,000,000 pounds, which exceeded by 26,000,000 pounds the amount received from out-of-state sources. W.D.S.

- 71. Change in Color of Winter Milk Natural.** W. E. KRAUSS, Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Agr. Exp. Sta. Weekly Press Bull. No. 22, Sept. 16, 1937.

No concern need be felt by the consumer over the progressive decrease in the yellow color of milk during the winter. This is a natural change resulting from the cows' decreased intake of carotene from the types of roughage usually available for winter feeding. In order to compensate for this drop in yellow color and hence vitamin A activity it is suggested that more milk be consumed, that the use of other vitamin A-rich foods be increased, or that standard vitamin A preparations be considered. W.E.K.

- 72. The Influence of the Feed Given to Dairy Cows on the Production of Milk of Good Industrial Quality.** H. L. BERAUD, J. M. ROSELL, AND JULES TURGEON, Dept. of Bacteriology of the Agr. Faculty, Montreal, Canada. *Le Lait* 16, 160, p. 1068, Dec., 1936.

Milk of undesirable odor and taste is produced when the cows consume garlic, onion, turnip, leek, wormwood, potatoes, lupin, clover, sugar beet waste, etc. If the consumption of such material is four hours or more before milking, the possibilities of the milk being undesirable in flavor are considerably reduced. However, it is best to eliminate all such feeds as well as molded or spoiled feeds from the ration. Feeds which increase the per-

centage of oleine in the butterfat produce a soft butter. Among such feeds are linseed and soya cake, corn gluten, oats, wheat bran, and green forage. On the contrary, the feeds that reduce the percentage of oleine have an opposite effect, *i.e.*, they produce a butter that is too firm or too dry. Among such feeds are potatoes, wheat of India, corn silage, sugar beets, cottonseed cake, hay and alfalfa. By choice of the proper feeds, summer and winter butters may be given the desired physcial properties. Certain ensilages and sugar beets alter the fermenting predisposition of milk in such a way as to render it nonadaptable for manufacturing purposes. Such milk should be used for immediate consumption and not for the manufacture of butter and cheese. For the production of certain types of cheese it is necessary to control carefully the feed of the cow. In this connection the Swiss regulations for forage and forage management are given. A.H.J.

Other abstracts of interest are numbers 49, 53, 54, 56, 58, 59, and 60.

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## ABSTRACTS OF LITERATURE

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# ABSTRACTS OF LITERATURE

## BACTERIOLOGY

- 73. A New Microscope with Photographic Chamber.** A. KARSTEN. *Le Lait* 17, 164, p. 337, April, 1937.

A microscope is described in which one can simultaneously observe the object and photograph it. Application of the instrument to photographing bacterial slides and plates and filtration fibers is discussed. A.H.J.

- 74. Dyes and Methods as They Affect the Methylene Blue Test.** J. M. FRAYER, Univ. of Vermont, Burlington, Vt. *Vt. Agr. Exp. Sta. Bul.* 424, June, 1937.

Three methods of conducting the methylene blue reduction test were compared, using 50 to 100 replicate tests of each sample being made.

1. Substantially as directed in the sixth edition of "Standard Methods of Milk Analysis."

2. Essentially, according to proposed changes to be embodied in the seventh edition of "Standard Methods for the Examination of Dairy Products."

3. Fundamentally, the English modified method as suggested by Wilson *et al.*

The data in hand suggest that the English method, which prescribes the careful inversion (not shaking) of each tube at 30-minute intervals, results in shortening the reduction times as well as in rendering them significantly more reliable as indicators. It is commonly recognized that the accuracy of the test as usually operated is impaired by the rising of the bacteria to the surface as the creaming process proceeds. The modified test yields more uniform bacterial dispersion brought about by frequent inversion.

The reduction times were much the same when methylene blue chloride and methylene blue thiocyanate were used in approximately equal concentrations. Significantly longer reduction times resulted when these dyes were used in concentrations of 1:300,000 than when used at concentrations of 1:700,000. J.M.F.

- 75. Bacterial Content of Milk as Affected by the Use of Different Plating Methods.** ALEC BRADFELD, Univ. of Vermont, Burlington, Vt. *Vt. Agr. Exp. Sta. Bul.* 417, April, 1937.

In this study tryptone-glucose-skimmilk agar was compared with standard agar at 32° C. (89.6° F.) and at 37° C. (98.6° F.) incubation temperatures using representative samples of both raw and pasteurized milks carry-

ing heterogeneous bacterial flora as well as milks contaminated from specific sources.

Greater increases in counts were attributed to lower incubation temperatures than to change in medium composition, although modified medium gave higher counts than did the standard.

The modified medium developed larger colonies and was more opaque which made counting easier and doubtless more accurate.

The modified medium plus 32° C. (89.6° F.) incubation gave higher counts in nine-tenths of the samples of raw and pasteurized milk, the latter giving three times as great an average percentage increase as the former.

Organisms derived from the udder, feces, hair, etc., apparently grew almost as well on the standard as on the modified medium. Those developed because of inadequate cooling or derived from unsterile utensils, feed and stable dust grew in significantly greater numbers at the lower incubation temperatures on both media but more particularly on the modified.

Skimmilk is an important ingredient in media, especially for plating pasteurized milk.

Dry milk solids, made by either the spray or flake process, appear to be as satisfactory for this purpose as does skimmilk, and may be added to the media in powder form together with the other ingredients.

On the whole, the Bowers-Hucker medium incubated at 32° C. (89.6° F.) proved to be more accurate and consistent than the standard as a means of estimating the number of bacteria in milk, and it is believed that milks of good quality will not be seriously penalized by its use as a basis of grading.

J.M.F.

**76. Starters.** W. RITTER AND TH. NUSSBAUMER. *Schweiz Milchzeitung* No. 77, 1936.

After a short survey of the bacteriology of cream-acidifying cultures, the different methods for testing starters are discussed. Besides the test for bacterial pureness according to Burri's smear culture method, the following determinations are of first importance: the degree of acidity, the volatile acids, acetoin + diacetyl, eventually of carbonic acid and, above all, smell and flavor. Of the different methods of determining acetoin (acetylmethylcarbinol) + diacetyl, the most simple and most positive method is that of Hammer, but with an addition of  $\alpha$  naphthol.

Starters may be cultivated for a long time without undergoing remarkable changes. However, an alteration may suddenly take place which is characterized by a strong increase of the amount of volatile acids and a simultaneous decrease of acetylmethylcarbinol and diacetyl. Attempts to bring on this change artificially were not successful.

Experiments on the methylene blue reduction time of starters in milk pasteurized in different ways gave shorter reduction-times for milk sub-

mitted to high pasteurization than for milk submitted to holding pasteurization. Likewise small amounts of copper (2 mg. per litre) produced a much more prolonged reduction-time in milk submitted to holding pasteurization than in milk submitted to high pasteurization.

In conclusion some experiences made with a starter consisting of only one organism (acidifying as well as acetoin-+diacetyl-forming) are made known. W.R.

## BUTTER

**77. Actual Methods Used in the Making of Danish Butter.** J. M. ROSILL, Dept. of Bacteriology of the Agr. Faculty, Montreal, Canada. *Lait* 17, 161, p. 1, Jan., 1937.

Danish butter is characterized by its very desirable flavor. The flavor is obtained by use of high quality of cream in churning, by the use of butter culture and by proper care in all the culturing and churning operations. Neutralization of cream is not practiced although some washed cream may be used. Deodorization and removal of gases from cream is practiced in many creameries. The acidity at which the cream is churned is variable and is determined by the fat content of the cream to be churned. Details of the various steps in the manufacturing of Danish butter are reviewed.

A.H.J.

**78. A Holding Test at Room Temperature as an Indication of the Keeping Quality of Butter.** D. H. JACOBSEN. *So. Dak. Bul.* 308, 1937.

Interest is manifested in some means of determining or predicting the keeping quality of butter. Trials were carried out in which small samples were held at room temperature and the resulting flavor changes compared with those occurring at lower temperatures. Results obtained have indicated a need for further study.

This work included 40 churnings in which culture was used (15 salted and 25 unsalted) and 50 non-culture churnings (16 salted and 34 unsalted).

Particular attention was given to kind and extent of flavor deterioration and to kinds and numbers of bacteria which were present and which developed on holding.

Samples were held at 0°, 5°, 15°, and 21° C. The cream used in making the butter graded from sweet cream to 0.60 per cent acidity and samples were taken between September and June.

Results showed that a "holding test" consisting of holding small portions of unsalted butter for 7 to 10 days at room temperature (21° C.) gives useful information on the keeping quality of such butter at lower temperatures. The salted butter did not show flavor deterioration except for tallowiness within 7 days at 21° C., therefore no information was obtained on

the value of a holding test for the prediction of keeping quality of salted butter. The increases in numbers of total bacteria and the flavor deterioration in unsalted non-culture butter were much more extensive than in the unsalted culture butter held under similar temperature conditions. Salt in butter at the rate of 2.5 per cent effectively prevented the growth of lipolytic and proteolytic bacteria under the conditions of this investigation.

C.C.T.

**79. Fishy Flavor of Butter and the Means of Checking It. W. RITTER.**  
Schweiz. Milchzeitung No. 95-98, 1936.

A short report is made on the forthcoming of fishy flavor in butter and the means of preventing it. In Switzerland as well as elsewhere fishy flavor in butter could be kept down by omitting to acidify the pasteurized cream. It must, however, become possible to prevent fishy flavor also in butter made of acidified cream. For this reason, and in addition to Swedish and a great number of Swiss experiences, in the central plant at Lucerne were carried on during a certain period experiments on the influence of pasteurization-temperature on the forthcoming of fishy flavor in butter. From these experiments it followed that, if the cream was heated at 78, 80 and 82° C. in the flash pasteurizer, fishy flavor developed regularly after some time in the butter, although it was not salted. If the pasteurization-temperature was brought to 86° C., fishy flavor appeared in the butter after some time of storing in about half the cases. If the cream was heated at 90° C., fishy flavor was not found in the butter even after a storage of 10 weeks. The experiments were carried out with two maturators of 3000 litres of cream each. In order to keep down the cooking flavor of the cream, the difference between the temperature of the heating-water and that of the cream must be as slight as possible. This may be attained by increasing the number of plates, especially in the heat recuperating compartment. From different analogous cases it follows that the favorable action of an increased pasteurization temperature is due to the development of antioxidizing substances in the cream, not to a more radical destruction of the germs.

The results obtained at Lucerne respecting the influence of pasteurization temperature on the development of fishy flavor in butter are in agreement with other experiences concerning the keeping quality of Swiss butter made of acidified cream.

W.R.

**80. Investigations on the Phenomena Occurring During the Cooking of Butter (Ghee-manufacturing). W. RITTER AND T. NUSSBAUMER.**  
Schweiz. Milchzeitung 7 to 11, 1937.

It was found that the butterfat obtained by evaporation of the water at high temperature contains lecithin. On the other hand, the butterfat ob-

tained by liberation of the buttermilk at a low temperature is free from lecithin. In the first case an intense foaming takes place a second time towards the end of the cooking process. In this moment the lecithin passes from the cooking residuum into the foam in the form of brown flakes and is dissolved in the hot fat. During the cooling it is again precipitated as a brown, more or less flaky or slimy sediment. Fat which is free from lecithin becomes white when overheated. The presence of lecithin seems to be important also for the constancy against oxidation and it may equally delay to a certain extent the crystallization of the fat.

Butterfat is able to dissolve water. Fat that has been saturated with water at increased temperature becomes turbid when cooled, owing to the liberation of the water in the form of finest drops. The solubility of the water in the fat increasing with the temperature, it is difficult to remove the last traces of water without heating under reduced pressure or to long heating under ordinary pressure. Therefore such a butterfat contains generally still about 0.1 per cent of water. This water content may be somewhat reduced by filtration, etc.; it seems to be important for the reaction against oxidation and for the corrosion of the cans made of tinned iron. Especially in the latter case the way in which the liberated water is distributed becomes very important. The crystallization of the fat is different according to the kind of cooling. The fat ought to be as fine-grained as possible, as it is obtained by inoculating fat warmed at 30 to 33° C. with solid fat and keeping the mixture for some time at the above mentioned temperature. Further details on this subject will be given later on. W.R.

Other abstracts of interest are numbers 73, 76, 83, 84, and 95.

## CHEESE

81. Research on a Secondary Odiferous Fermentation or Gray Decay of Gruyere and Emmental Cheese. W. DORNER AND MARG. THOENI, Federal Dairy and Bacteriological Inst. at Liebefeld-Berne. *Le Lait* 17, 166, p. 561, June, 1937.

Odiferous fermentation or gray decay of cheese is a defect which appears 4 to 5 months or longer after the manufacture of the cheese. It is first evident as the pH of the cheese rises. This is eventually accomplished by a change in the odor and taste. At the end of a few weeks the odor becomes putrid and a grayish color develops. The cause of the fermentation was found to be *Bacterium proteolyticum* n. sp. When the milk used in cheese making contained as many of these organisms as 10 per cubic centimeter, secondary odiferous fermentation ensued. The properties of cheese that has been badly infected with the organism are a high pH value, a bad odor, a gray color or dark spots, and the liberation of gas. A.H.J.

82. **The Industrial Control of the Fat Content of Cheeses.** JEAN PIEN, Dir. of the Lab. of Farmer's Union, Paris, France. *Le Lait* 17, 166, p. 569, June, 1937.

Methods of determining the fat content of cheese are (1) dissolving the cheese in an acid or alkaline solution and extracting the solution with ether; (2) grinding the cheese with sand or an inert mineral salt and extracting with ether; and (3) dissolving the cheese in a stannous chloride solution and then determining the fat by the Gerber-Roeder method. The first method was considered impractical for use in the industrial laboratory. The second method was considered satisfactory and superior to the third method as far as accuracy of results was concerned. The Gerber-Roeder method was considered the most rapid as a control method but the results have the possibility of being inaccurate to the extent of  $\pm 1$  per cent. A.H.J.

Other abstracts of interest are numbers 76 and 95.

## CHEMISTRY

83. **The Determination of the Diacetyl in Butter.** JEAN PIEN, JACQUES BAISSÉ, AND ROBERT MARTIN. Dairy Lab. of Farmers Union, Paris, France. *Le Lait* 17, 167, p. 675, August, 1937.

Of 130 samples of butter investigated 11 per cent contained diacetyl between 1.0 and 0.5 milligrams per kilogram. All the butters which were high in their diacetyl content were considered very desirable in flavor and aroma. However, many samples which were very low in diacetyl were also excellent in flavor and aroma. This would indicate that other butter constituents besides diacetyl are responsible for desirable flavor and aroma in butter. Since no butters were found in which the diacetyl naturally present exceeded 1.0 milligrams per kilogram, it may be judged that diacetyl has been added to butters containing larger quantities of diacetyl than this. The addition of diacetyl to the extent of 20 milligrams per kilogram would not be practiced as such addition affects deleteriously the flavor and keeping quality of the butter. The addition of 10 milligrams per kilogram gives a flavor that is somewhat too strong but this quantity of diacetyl might find use in the butter industry. The use of 1 to 5 milligrams per kilogram improved the flavor and aroma of butter and caused no deleterious effects in butter stored for 8 days at 20–25° C. (68–77° F.). Such butter had a more desirable flavor and aroma than the control butter containing no added diacetyl.

In determining whether diacetyl has been added to butter, the quantities sought should, therefore, be between 1 and 10 milligrams per kilogram. Various methods for determining this quantity of diacetyl were investigated. The method adopted consists in the reaction between diaminobenzidine and diacetyl which yields diphenylquinoxaline. This compound is yellow in

solution and the diacetyl can accordingly be determined by a comparison of the yellow color with standards made with potassium dichromate. The diacetyl is removed from the butter in a special distillation apparatus which allows its concentration in 10 cubic centimeters of liquid. To the 10 cubic centimeters of distillate from the butter is added 0.5 cubic centimeter of a 2.5 per cent aqueous solution of diaminobenzidine (freshly prepared). After agitation 0.5 cubic centimeter of concentrated hydrochloric acid is added. After shaking again, color comparison can be made in about two minutes. In order that the color be stable, it is necessary to use the reagents in the proportions given. In working with 100 grams of butter, diacetyl may be determined in amounts as low as 0.5 milligram per kilogram. A.H.J.

**84. Detection and Determination of Diacetyl According to Pien, Baisse and Martin.** W. RITTER AND THS. NUSSBAUMER. Schweiz. Milchzeitung No. 103, 1936.

The method of diacetyl determination according to Pien, Baisse and Martin is based on quinoxalic condensation of diacetyl (as Diketon) with diamins, in the present case m-p. toluylendiamin. Thereby a yellow dimethylquinoxalin is formed that can be determined colorimetrically. The method gives pure yellow colorings if pure reagents are used as ever fresh solutions. On the other hand, the method presents a disadvantage, that is to say: acetylmethylcarbinol (acetoin) which is present in the cultures must be oxidized first and has to be distilled in the form of diacetyl, just in the same way as in the nickel-dimethyl-gloxin-method. This is not necessary in the Voges-Proskauer-method carried out according to Hammer by means of Kreatin and solution of sodium hydroxide, to which some  $\alpha$ -naphthol may be added. W.R.

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

**85. The Influence of Irradiation on the Content of Vitamin A in Concentrated Milks.** G. GENIN, Paris, France. *Le Lait* 17, 164, p. 368, April, 1937.

Irradiated evaporated milk was found to have the same vitamin A content as the same evaporated milk not irradiated—*i.e.*, 4.28 units of vitamin A per gram of milk. Ultra-violet irradiation of the milk accordingly did not reduce the vitamin A content of the milk. A.H.J.

**86. Physico-Chemical Modifications of the Constituents of Milk in the Course of Concentrating in the Presence of Sugar Syrup.** MICHAEL POLONOVSKI. *Le Lait* 17, 167, p. 699, July–August, 1937.

Sweetened condensed milk was found to yield excellent results in infant feeding. An explanation was sought in the physico-chemical changes oc-

curing during the manufacture of the milk. Sweetened condensed milk had a higher viscosity and a higher surface tension and a decreased flocculability than sweetened pasteurized milk. The buffer value underwent only a small variation and the change was inconsistent. It is difficult to associate any of these changes with the better utilization of milk in infant nutrition. The decreased flocculability with associated softer and less rapidly forming curd is probably the most significant factor. The changes in surface tension and viscosity are probably associated with more profound changes in the protein molecule and their relationship to infant nutrition has not been studied.

A.H.J.

**87. An Automatic Procedure for the Making of Milk Powder.** G. GENIN, Paris, France. *Le Lait* 17, 165, p. 488, May, 1937.

The Gray Jensen process for the manufacture of milk powder is described.

A.H.J.

**88. A New American Plant for the Manufacture of Lactic Acid.** G. GENIN, Paris, France. *Le Lait* 17, 163, p. 259, March, 1937.

The manufacture of lactic acid and sodium and calcium lactates from whey is described. The milk sugar in the whey is fermented to lactic acid by a mixed culture of *Lactobacillus Bulgaricus* and a mycoderm. When the acidity of the fermenting whey reaches 1.3 per cent, lime is added to neutralize the acid and the fermentation allowed to continue. It is usually necessary to add lime 4 times during the fermentation of a tank of whey. About 48 hours are required to convert the lactose in the whey to lactate. At the end of the fermentation, an excess of lime is added and the mass heated to 90° C. (194° F.) in order to precipitate the lactalbumin. The precipitate is removed by passing the liquid through a filter press. The filtered liquid is clarified by the use of activated carbon and then concentrated and cooled whereupon the calcium lactate crystallizes. The calcium lactate may be further purified by dissolving, re-decolorizing, concentrating, and re-crystallizing. In order to prepare lactic acid the calcium lactate solution is treated with sulphuric acid whereupon calcium sulphate precipitates. The lactic acid is obtained by filtering off the calcium sulphate. By modifying the procedure lactic acid of 22 and 44 per cent concentration may be obtained. Sodium lactate is prepared by neutralizing the lactic acid with caustic soda.

A.H.J.

**89. The World Production of Casein.** G. GENIN, Paris, France. *Le Lait* 17, 168, p. 605, June, 1937.

World production of casein was 60,000 tons in 1932 and 70,000 tons in 1936. The chief uses to which the casein is put are as plastic material, glue,

paper coating, in insecticides, in the textile industry, in casein paints and as a food material. In the United States two-thirds of the casein produced is used in the paper industry. The United States, France and Argentina are the chief countries producing casein, these countries each producing about 18,000 tons annually. A.H.J.

Other abstracts of interest are numbers 73, 74, 75, 90, 91, 92, 93, 95, 104, 108, 110, 115, 118, and 119.

## DISEASES

**90. Mastitis—Tests for Detecting Its Presence in Milk.** K. G. WECKEL, Univ. of Wisconsin. *Milk Dealer* 26, 12, p. 70, Sept., 1937.

The author relates the effect of mastitis on milk and describes some of the tests for determining the presence of mastitis in the herd. C.J.B.

**91. Mastitis. V. The Presence of Mastitis Streptococci in Bovine Mammary Tissue.** G. J. HUCKER, N. Y. Agr. Exp. Sta., Geneva, N. Y. *Tech. Bul.* 241, Jan., 1937.

"In order to determine the prevalence of mastitis streptococci in bovine mammary tissue, 46 udders were aseptically removed from cows and virgin heifers and cultured. Only udders were studied in which no evidence of infection could be noted by gross examination of the udder prior and subsequent to removal. Inasmuch as only mastitis-free cows from the standpoint of physical and post-mortem examination were used, a considerable number of animals were observed to secure the 46 used in the present investigations.

A study of 24 udders aseptically removed and cultured from cows known to be free of mastitis and to have passed through one or more lactation periods shows that all contained mastitis streptococci.

A similar study of 21 udders removed from virgin heifers and calves indicated that less than half contained mastitis streptococci in the mammary tissue." G.J.H.

**92. Mastitis. VI. The Effect of Feeding Irradiated Yeast on the Resistance of the Udder to Bovine Mastitis.** G. J. HUCKER AND MARION SNYDER REED, N. Y. Agr. Exp. Sta., Geneva, N. Y. *Tech. Bul.* 243, July, 1937.

"To determine the effect of irradiated yeast on the resistance of dairy cows to udder infections, 116 cows in four dairy herds were studied. Fifty-one were fed irradiated yeast in the grain mixture. In three herds 9 ounces per day per cow and in one herd 4½ ounces per day per cow were fed.

Prior to the beginning of the feeding of the yeast, weekly quarter samples from all cows were subject to laboratory examination over a 4-month

period to determine the amount of mastitis infection present. Examinations were made for presence of mastitis streptococci, number of leucocytes per cc, reaction to brom thymol blue, and physical appearance of the milk. Subsequent to the initiation of the yeast feeding, similar samples were secured for approximately 20 months. In all, the observations were made on the experimental herds over a period of 2 years.

In general the feeding of irradiated yeast was found to have no significant effect upon the resistance of the udder to the invasion of mastitis streptococci. Depending upon the index of infection used, from 10 to 13 per cent more of the infected yeast-fed cows showed an improvement than was found in the case of the infected cows not fed yeast.

No prophylactic effect was found by the addition of irradiated yeast to the diet of mastitis-free cows." G.J.H.

**93. Mastitis. VIII. The Use of a Specially Prepared Vaccine in an Attempt to Control Bovine Mastitis.** G. J. HUCKER AND P. A. HANSEN, N. Y. Agr. Exp. Sta., Geneva, N. Y. Tech. Bul. 245, July, 1937.

"A study was made of the possible prophylactic and therapeutic action of a vaccine prepared from stock and freshly isolated herd strains of *Streptococcus agalactiae* Lehmann and Neumann. Injections of milk were used in connection with the vaccines.

One hundred and two animals in four herds were studied over a period of 2 years, 45 being vaccinated and 57 being retained as untreated controls.

Weekly quarter samples were secured for 4 to 7 weeks prior to vaccination and weekly quarter samples for 3 months and monthly quarter samples for approximately 14 months subsequent to vaccination were examined in the laboratory for presence of mastitis streptococci, number of leucocytes per cc., reaction to brom thymol blue, and physical appearance of the milk.

It is concluded that vaccines prepared from stock and freshly isolated strains of *Streptococcus agalactiae* give no evidence of increasing the resistance of dairy cattle to mastitis.

Similar vaccines were found to have little or no therapeutic action in the treatment of latent and chronic udder infections. Neither the prophylactic nor therapeutic action of this vaccine was stimulated by the use of simultaneous intramuscular injections of milk." G.J.H.

## FEEDS AND FEEDING

**94. Animal Nutrition.** LEONARD A. MAYNARD. McGraw-Hill Book Co., New York, 1937, pp. 483. Price \$4.00.

This book, according to the preface, is based upon twenty years experience in teaching the principles of animal nutrition to graduate and under-

graduate students at Cornell University. It was written primarily to give a systematic knowledge of these principles and not to give specific directions for feeding farm animals. Such being the case the book must impart more knowledge about feeding rats than cows as so many of the facts of nutrition were gained in this way.

The various chapters of the book deal with the expanding field of nutrition, the animal body and food, physical bases of life processes, carbohydrates and their metabolism, lipids and their metabolism, proteins and their metabolism, inorganic elements and their metabolism, vitamins, determination of digestibility, nutrition balances, nutritive energy, maintenance, growth, reproduction, lactation, and work production. A considerable portion of the book deals with chemistry and physiology. For example, the chapter on carbohydrates devotes eight pages to the classification and chemistry of carbohydrates of special interest in animal nutrition, and the chapter on lactation is much concerned with the anatomy of the udder and the physiology of milk secretion. A knowledge of this scientific background is essential to a thorough understanding of the general subject of nutrition.

The author has succeeded particularly well in developing an interesting style. Innumerable facts on the problems confronting the field of nutrition, on special applications to human nutrition, on pioneer contributions of many individuals and the author's interpretations give special interest that even the college student may read certain passages without the oppressive remembrance that he is studying. This book is very well organized and the subject-matter has been carefully selected.

A.C.D.

**95. The Weston-Levine Vitamin Chart, Seventh Revision, Sept., 1937.**

ROE E. REMINGTON, Medical College of the State of South Carolina,  
Charleston, S. C.

This chart, originally published in 1931, has been revised annually. By careful selection and condensation of authentic information it summarizes the latest available data as to the composition and properties, principal functions, results of deficiency, and food sources of the vitamins.

In the seventh revision note has been taken of the most recent progress in vitamin research, particularly in the identification of the various factors of the B complex, including Williams' synthesis of vitamin B<sub>1</sub>. Flavin is substituted for vitamin G and the P P factor added. The results of vitamin deficiency have been classified as specific and non-specific, and the list of food sources checked with results appearing since the last edition. The list of references has been limited to those most likely to be of value to students of the subject.

The chart is offered for sale by the author.

R.E.E.

96. **Bacteriological Investigations About Ensiling Green Forage with the Addition of Acid Whey, Skimmilk or Sugar.** J. VAN BEYNUM AND J. W. PETTE. Report of the Experimental Dairy Farm, Hoorn, Holland, pp. 1-38, 1937 (English summary).

This work is a continuation of studies reported in 1935 to improve grass silages by cultured whey, skimmilk, or buttermilk.

The pH is the principal controlling factor in silage quality. It must be 4.2 or less. If higher *clostridium tyrobutyricum* attacks the lactic acid converting two molecules into one of butyric acid, thereby increasing the pH and promoting spoilage.

The lactobacilli are more important than the lactic streptococci as the latter do not produce enough acid. Whey added to the grasses is beneficial by increasing the moisture content to facilitate the exclusion of air and by increasing the sugar content for lactic fermentation. A.C.D.

97. **Experiments with a Practical Silage Problem Dealing with the Addition of Dairy Wastes or Sugar.** E. BROUWER. Report of the Experimental Dairy Farm, Hoorn, Holland, pp. 39-102, 1937 (German summary).

It has been established that a knowledge of pH of silage is invaluable. For example, a pH value of 4.5 or above indicates poor quality, a value range of 4.0 to 4.5 places the silage in a doubtful class, while good fermentation feeds have a pH of 4.0 or lower. However, a silage with a pH value of less than 4.0 may be detrimental for cheese purposes. This is especially true when certain organisms and spores are present.

It has been learned that the sense of smelling is most valuable in classifying silage. Sweet odors are desirable and mild clean acid odors are most objectionable. It is even possible to classify a silage as good that has a fruity odor. A rotten or butyric acid odor must not be present. The odor of certain esters in silages places them in a doubtful class. Classifying silage on the basis of odor is regarded as being comparable or better than doing so with numerous analyses as a guide.

The experiment dealt with the addition of 3 to 10 per cent of whey to silage. Wooden, stone and iron silos were used. With this procedure less than 6 per cent of the 121 silos studies produced a silage with a pH value of less than 4.0 and 62 per cent of the samples had pH values greater than 4.5. Butyric acid and ammonia were present in many samples. Many samples had a very bad odor which was readily transmitted to the milk or its products.

The author points out that the identification of organisms found in silage is not important, but that a knowledge of the kinds that grow in the silage is the important item.

Comparable results were obtained with silos and pit silos. The argument that a tall sweet silo will guarantee good silage is a fallacious assumption.

The ammonia content of grass silage increased greatly with rising pH values. At a pH of 4.0, 8 per cent of the total protein was ammonia; at a pH of 5.0, 24 per cent of the total protein was ammonia. This same relationship held for other silage feeds.

Silage with a pH of 4.0 contains very little butyric acid but with a rise in pH the butyric acid content increased. This held in a lesser degree with other silage feeds than with grasses.

Bean silage was not eaten freely by cattle. Various silages gave comparable results from the point of view of the health of the animals.

Nineteen trials were conducted with the addition of 0.5 per cent of sugar to the grass, 15 earth silos were used, 3 silos and one Feimen (stacked). Only 4 trials gave pH values of 4.0 or lower, 9 gave pH values higher than 4.5.

Whey or 0.5 per cent of sugar added to silages did not improve it according to 140 trials made in a practical way. J.C.M.

**98. Bacteriological Examination of Silages Prepared with Addition of Whey, Skimmilk and Sugar on Farms in Netherland.** J. VAN BEYNUM AND J. W. PETTE. Report of the Experimental Dairy Farm, Hoorn, Holland, pp. 103-157, 1937 (English summary).

There are three types of grass silage made without the addition of inorganic acids. Type A silage has not heated in fermentation, hence a lactic acid fermentation lowered the pH below 4.2 because plenty of sugar was present, and the silage is of good quality. If the added sugar was not well distributed there will be a bad spotty butyric acid fermentation in the low sugar areas. In Type B silage the sugar content was too low and fermentation ceased above pH 4.2. Then *clostridium tyrobutyricum* fermented the lactic acid into butyric and the old silage became putrid. Type C silage heated during fermentation and destroyed both lactic and butyric bacteria. It is much like spontaneously heated hay.

Most grass silages with added whey are Type B for the sugar content is too low. Their pH gradually increases with age to 6 and the quality decreases. By added sugar the pH may be made sufficiently low to keep well. The acid distribution is better in sugar silage than in A. I. V. silage. Type C silage should not occur when whey is used.

In 1934 grass silages with one per cent added sugar, with and without cultures, did not keep well. Best results have been secured recently by adding both whey and sugar or an increased amount of sugar.

A.C.D.

**99. Artificial Hay-making.** G. GENIN, Paris, France. *Le Lait* 16, 160, p. 1061, Dec., 1936.

Young grass is dried in a heated chamber to yield a hay that is high in protein, vitamin A, ash constituents, and other food elements necessary for

good growth of young animals and for high milk production in dairy cows. Four cuttings of young grass per year materially increase the yield of hay. Because of the intensive cropping, the use of fertilizer is recommended. The feeding of artificially dried young grass makes it unnecessary to use more expensive concentrates in the ration. The cost of an artificial drying may be reduced appreciably by exposing the young grass to sunshine and then finishing the drying in the artificial drier. This allows some loss in feed value but dried young grass is still of excellent quality. A.H.J.

**100. Virgin Rice Bran in the Feeding of Dairy Cows . . . Experiments of 1936.** TOLESFORO BONADONNA, School of Zootechnolgy of the Faculty of Vet. Med., Milan, Italy. *Le Lait* 17, 170, p. 1025, Dec., 1937.

Virgin rice bran when pure and properly cared for, *i.e.*, devoid of all foreign matter capable of adulterating it, is an excellent and economical feed for the nutrition of dairy cattle. In the proportion of 40 to 80 per cent of the ration (from 1 kilogram to 1.5 kilogram and more per day) rice bran exerted no harmful effect on the health of the cows even during hot weather. From the point of view of cheese making, "Reggio" type, rice bran, provided it was pure and not fermented, did not have any harmful effect. The maturing of the cheese was, however, more rapid when rice bran was included in the ration of the cow. Rice bran also influenced certain properties of the butter, but the change depends on the nature and composition of the other feed given with the rice bran. A.H.J.

## ICE CREAM

**101. The Bacteriological Quality of the Ice Cream Supply for a Small City.** M. W. YALE AND R. C. HICKEY, N. Y. Agr. Exp. Station, Geneva, N. Y. Tech. Bul. No. 248, Sept., 1937.

With the exception of three or four of the larger cities in New York State, but little work is being done by municipalities on ice cream sanitation, and knowledge in respect to sanitary quality of ice cream and ice cream ingredients is quite inadequate.

A bacteriological study was made of the ice cream supply for a small city since it was believed that the results would be of general interest and applicable to the situation in many other municipalities.

Both total and coliform counts were determined for 77 process samples from 7 local plants, 137 ice cream samples from 12 retail stores, and 36 dipper water samples from 18 establishments. Process samples demonstrated that either gelatin or color was excessively contaminated at four plants and cream at one plant. Freezing equipment was in poor sanitary condition at two plants.

Coliform counts were more sensitive than either standard nutrient agar or tryptone agar counts in revealing contamination by ice cream dippers and dipper waters which were in poor sanitary condition in the majority of instances.

Three of the 12 manufacturers had all standard agar plate counts of store samples of vanilla ice cream under 100,000 per gram. Eight manufacturers had an average (logarithmic) standard plate count under 100,000 per gram. The average count of 112 store samples was 59,800 per gram, a higher average than usually found in cities and states where bacteriological control is exercised. In the case of 9 of the 12 manufacturers, there had been no previous bacteriological control of their product. M.W.Y.

Other abstracts of interest are numbers 73, 74, 75, 78, 86, 95, 103, 113, and 119.

## MILK

### 102. The Use of a Tincture of Guaiacum in the Control of Pasteurization.

B. LEBOVITCH, Chief of the Lab. of the St. Hubert Dairy at Nancy, France. *Le Lait* 17, 165, p. 463, May, 1937.

Various chemical methods of determining the effectiveness of pasteurization were investigated.

A preparation consisting of 100 cubic centimeters of acetone, 1 gram of gum guaiacum, and with or without from  $\frac{1}{2}$  to 3 grams of guaiacol was considered to yield satisfactory results. The use of guaiacol made the reagent more sensitive. With  $\frac{1}{2}$  gram of guaiacol in the reagent, milks flash pasteurized at 76 and 77° C. (168.8 and 170.0° F.) showed positive reactions while when pasteurized at 78, 79, and 80 (172.4, 174.2 and 176.0° F.), the reaction was negative. However, when 3 grams of guaiacol are used in the reagent only the milks flash pasteurized at 79 and 80° C. (174.2 and 176.0° F.) show negative results. These and other data indicate that peroxidase in the milk is not destroyed even on flash pasteurizing at 79° C. (174.2° F.). On using  $\frac{1}{2}$  gram of guaiacol in the reagent, the milks which gave negative reactions when flash pasteurized at 78, 79, and 80, (172.4, 174.2, and 176.0° F.) gave positive reactions when 1 per cent of raw milk was added. Where flash pasteurization is practiced, it is considered that this test supplies a control measure that will allow the production of a truly hygienic milk.

A.H.J.

### 103. The Bacteriological Standard for Pasteurized Milk. A. L. PROVAN. *Milk Industry* 17, 6, p. 45, Dec., 1936.

The growth of many bacteria found in pasteurized milk can be greatly increased by the addition of one per cent milk to the agar media.

Memo. 139/Foods (Jan. 1937) issued by the Ministry of Health gives details of the test to be used for control purposes. The new order states that pasteurized milk shall contain not more than 100,000 bacteria per ml. as determined by the colony count on a medium which contains one per cent of milk. The new media now recommended by the Ministry of Health (1936 order) is:

Yeastral ... .	3 gr.	Whole milk . . .	10 gr.
Peptone ... .	5 gr.	Water . . .	1,000 gr.
Agar ... .	15 gr.		

Dr. Provan in anticipating this order in 1935 used a media which was slightly different from that now recommended by the Ministry of Health in order to determine the effect that the addition of milk might have on the results obtained on pasteurized milk.

The media he used contained 3.0 gr. of Lemco in place of the yeastral and 10.0 gr. of skim milk in place of whole milk now recommended. In comparing his media with the Standard Agar (1923 order) he found that the milk agar often gave greater counts than did the plain agar. The higher the count on the plain agar the greater the percentage of samples showing a decidedly higher count on the milk agar.

There is some evidence that heat resistant organisms present in the raw milk will be of greater importance when milk agar is used for determining the count.

L.H.B.

#### 104. The Numbers and Kinds of Bacteria in Aseptically Drawn Milk.

H. R. THORNTON AND N. J. STRYNADKA. Twenty-Fourth Ann. Report of the Intern. Assoc. Dairy and Milk Inspection, p. 178, Oct., 1935.

Milk was aseptically drawn into sterile flasks from 95 cows in 12 herds. Two were from fresh cows (within 48 hours after parturition) and were labeled colostrum milks. Five were from cows having mastitis and were labeled mastitis milks. Eighty-eight were milks normal in appearance. One thousand fields were examined per smear. Of the 88 normal samples of milk 31 contained less than 500,000 leukocytes per cc. and 57 contained more than 500,000 per cc.

The average Breed count for the 31 samples containing 500,000 or less leukocytes per cc. was 34,980 and the average plate count was 918 and the average Breed count for the 57 samples containing over 500,000 leukocytes per cc. was 204,515 and the average plate count was 36,625. No rod shaped bacteria were observed in any of the 95 samples of milk examined.

Only single cocci were found in 35.5 per cent of the low leukocyte count milks and in 15.8 per cent of the higher leukocyte count milks.

Groups no larger than diplococci were found in 38.7 per cent of the low leukocyte count milks and in 24.6 per cent of the high leukocyte count milks.

Streptococci (three or more in chain) were found in 16.6 per cent of the low leukocyte count milk and in 42.1 per cent of the high leukocyte count milk.

Clumps (three or more in non-chain formation) were found in 22.6 per cent of the low leukocyte count milks and in 26.3 per cent of the high leukocyte count milks. L.H.B.

**105. The Milk Supply of the Nation. Hygienic Production and Control.**

BEN DAVIES, Dir. of the Lab. for United Dairies, London, England.

Le Lait 17, 165, p. 449, May; 166, p. 591, 1937.

The importance of proper hygienic control of milk production is emphasized. The determination of the bacterial counts of pasteurized milk rather than raw milk is suggested as a control measure as in milks properly produced in equipment that is not satisfactorily cleaned, the bacterial count may even increase on pasteurization. The pasteurization of milk of every quality is recommended. A.H.J.

**106. The Utilization of Tubercular Milks. J. VERGE AND G. THIEULIN,**

Vet. School at Alfort, France. Le Lait, 17, 164, p. 348, April, 1937.

According to French law milks containing tuberculosis organisms or originating from animals afflicted with tuberculosis cannot be used for food of man or animals either as such or as manufactured products unless they are heated sufficiently to destroy the tuberculosis organisms. A.H.J.

**107. Colon Bacilli and Bacteriophages of Milk to be Consumed in the**

Raw State in Warsaw. IRENE LIPSKA, Municipal Inst. of Hygiene at Warsaw, Poland. Le Lait 17, 163, p. 236, March, 1937.

The colon bacilli of winter milk as well as of summer milk are such as are characterized by biochemical properties intermediate between the species *Bacterium coli commune* and *Bacterium lactis aerogenes*. The filtrates of summer milk were more active with respect to colon bacilli of milk and with respect to animal and human excrement and urine of those afflicted with kidney maladies than those of winter milk. There was observed an analogy in the activity of the milk for colon bacilli and for typhoid and paratyphoid bacilli. The greater extent of the activity of the filtrate from summer milk included 16 strains from 23 bacilli tests. The most frequently attacked were the colon bacilli from animal and human excrement; those of milk were more resistant, the most refractory being the pathogenic colon bacilli isolated from urine. A.H.J.

**108. The Production of a Clean and Healthful Milk on the Farm. A.**

TAPERNOUX, Prof. of Chemistry at the Vet. School at Lyons, France.

Le Lait 17, 163, p. 241, March, 1937.

The chemical and biochemical composition of milk is discussed. The production of a clean and healthful milk on the farm means the elimination of infected animals from the herd, the exercise of proper milking technique, and the maintenance of satisfactory sanitary conditions in the barn. A.H.J.

- 109. Actinization of Milk.** JEAN VIEILLY AND JEAN HARDER, Doctors of Veterinary Medicine of the Industrial Dairy at Grenoble. *Le Lait* 17, 166, p. 576, June; 167, p. 707, July–August, 1937.

Actinization is defined as a treatment with ultra-violet rays for the hygienic purpose of destroying dangerous organisms and the conservation of lactic acid organisms and for the nutritive purpose of increasing the vitamin D potency. The actinizer of Stoutz is described in which the milk is irradiated as it flows through quartz tubes. In the actinization of milk, it becomes sterile at the exposed surface proportional to the time exposed to the rays, bacterial colonies are destroyed, and chemical and physical modifications are imparted to the milk. Infants appear to tolerate actinized milk better than normal milk, the digestibility appears to be improved, and the vitamin D potency is increased. A.H.J.

- 110. The Preservation of Dairy Products by the Procedure of Hofius.** G. GENIN, Paris, France. *Le Lait*. 17, 167, p. 727, July–August, 1937.

Unpasteurized milk at about 8° C. (46.4° F.) is placed in a steel cylinder so that it is three quarters full. Oxygen under 8 atmosphere pressure is then introduced into the cylinder and the milk shaken. The valve on the cylinder is then opened and the oxygen pressure relieved. The outgoing oxygen carries with it any gases dissolved in the milk. Oxygen is again introduced into the cylinder until a pressure of 8 atmospheres is attached. The cylinder containing the milk is then placed at a temperature of 8° C. (46.4° F.) and shaken for 2 hours. If the pressure in the cylinder is still above 7 atmospheres the cylinder of milk is placed in storage at 3 to 6° C. (37.4 to 42.8° F.), otherwise the oxygen pressure is raised to 8 atmospheres before the milk is stored. The milk will keep and there will be no increase in acidity during a storage period of 14 days. If the milk is heated rapidly but not pasteurized before being subjected to the oxygen treatment it will keep for 4 weeks. When such milk is held for 42 days there will be a considerable development of off-flavor in the milk. If the storage temperature of milk treated by the Hofius process attains 18 to 22° C. (64.4 to 71.6° F.), the acidity will show an increase at the end of 3 days. Milk serum keeps less well by the Hofius process than whole milk. The off-flavor which develops in whole milk treated by the Hofius process is that of caramelization; in milk serum, a malt-like flavor. Peroxides have not been detected in milks treated by this process. A.H.J.

- 111. The Purification of Milk in the Dairy Industry. The Necessity for Filtration or the Clarifying Centrifuge. Advantages and Disadvantages.** J. GIROUX, Director of the Lab. for the Control of Milk and Chemical and Bacteriological Research of the Maggi Dairy Society. *Le Lait* 17, 164, p. 354, April, 1937.

The removal of foreign matter from milk by filtration or by the clarifying centrifuge is necessary to assure complete freedom from sediment. The centrifuge is more satisfactory than the filtration but filtration is a simpler and less expensive operation. Neither of the two methods of purifying milk remove significantly large amounts of milk constituents from the milk. The clarifying centrifuge remove about 80.5 milligrams per liter of milk of which the composition is 33.9 per cent dry matter, 2.09 per cent fatty matter, 22.23 per cent total nitrogen (as casein), 4.08 per cent carbohydrate, and 5.5 per cent ash. When calculated to the fluid milk these losses do not constitute a very large percentage. During the centrifuging the slime which is formed carries with it most of the impurities that have gotten into the milk. Neither the action of the centrifuge nor of filtration removes the bacterial flora from the milk. Centrifugation has the advantages of security, constant milk flow, possibility of working at low temperature, more effective removal of debris and animal cells and economy of operation. Filtration has the advantages of being simple, non-mechanical, and excludes air more effectively from the milk. Filtration has the disadvantages of requiring heated milk, of not removing suspended material so well, of requiring frequent replacing of the filtering tissue, and difficulty of cleaning. Before pasteurization or sterilization, the practice of both filtration and clarifying centrifugation of the milk or at least one of them is desirable.

A.H.J.

- 112. Consideration on Bulgarian Curdled Milk Prepared from Sheep's Milk.** I. KVATCHKOFF. *Le Lait* 17, 165, p. 472, May, 1937.

Various organisms that are used in making cultured milks are discussed. Methods of manufacturing cultured milk are given as is also the chemical composition of the finished products. Properly made Bulgarian curdled milk had excellent keeping quality. Such milk of the proper acidity will keep for a month or more when stored at low temperature. This makes it possible to ship the product made from sheep's milk to countries where sheep's milk is not available. Bulgaria has an annual production of about 2.5 million hectoliters of sheep's milk.

A.H.J.

- 113. The Influence of Metals on Milk.** W. RITTER. *Schweiz. Milchzeitung* No. 59-60, 1936.

After discussing prevailing experiences, the author reports about his own experiments. In order to make the influence of metals more evident, the

milk was submitted to holding pasteurization, cooled, and stored for 24 and 48 hours. Metals are more apt to produce tallowy flavor in milk when in contact with it during the holding pasteurization and not only during storage thereafter. Copper and all copper-alloys (brass, bronze, German silver, monel metal) develop tallowy flavor when the milk is treated in this way. Nickel leaves a vitiated, not distinctly tallowy flavor in milk. Aluminum, tinned and chromed material, and stainless steel do not affect the flavor of milk.

When determining the aldehyde-reductase (xanthine oxidase) in milk submitted to holding pasteurization in the presence of metals, the strongly damaging influence of copper and all its alloys becomes again evident. This influence is even exerted, though in a very slight degree, by tinned copper and tinned German silver. All these copper-containing metals are distinguished by a more or less prolonged reduction time, according to their yielding of copper.

The copper contents of a metal can also be detected by applying high pasteurization to milk treated with metals and submitting the milk to the peroxidase-reaction after cooling it. This procedure reveals again the presence of all copper-containing metals in milk by a rapid setting in of a positive reaction with dimethyl-p-phenyldiamin-sulphate,  $\alpha$ -naphthol and hydrogen-peroxide.

In a similar way, but more feebly, copper in all its alloys influences the nadi-reaction of the heated milk (dimethyl-p-phenyldiamin-sulphate and  $\alpha$  naphthol, without hydrogen-peroxide).

The non copper-containing alloys, even if they are able, as for instance nickel, to produce a vitiated flavor in milk, do not affect the aldehyde-reductase (xanthine oxidase), peroxidase- and nadi-reaction. W.R.

#### 114. The Influence of Bacterial Activity on the Peroxidase-Reaction of Copper-Containing Milk. W. RITTER. Schweiz. Milchzeitung 89, 1936.

Small amounts of copper in milk can be traced by heating the latter rapidly to 85° C. and submitting it afterwards to peroxidase-reaction with dimethyl-p-phenyldiamine-sulphate,  $\alpha$ -naphthol and hydrogen-peroxide. An important bacterial content of the milk is liable to influence this reaction in two different ways; Slackening it by reducing the easily reducible indophenol dye which is formed, or intensifying it by accelerating the nadi-reaction through different bacteria. The bacteria being for the most part killed by high pasteurization and a renewed infection being scarcely probable, this case presents a merely theoretical interest.

On the other hand an intense bacterial activity taking place before pasteurization is liable to exert a slackening influence on the peroxidase-reaction also after pasteurization, if there are only very small amounts of

copper. As soon as the copper content is but slightly increased, the copper reveals itself all the same, although somewhat slowly. Acid milk cannot be used; even milk which is not acid, but too ripe, is apt to prevent the setting in of the reaction. This phenomenon is probably due to the effect of certain chemical substances able to resist high temperature. It follows that only fresh milk or cream should be submitted to the peroxylase-reaction for the detection of copper. W.R.

**115. Control of Onion Flavors in Milk.** C. E. WYLIE, Univ. of Tenn. Milk Dealer 27, 1, p. 84, Oct., 1937.

The author briefly discusses the means of controlling onion flavor by (1) removing the onion flavor from milk, (2) getting the cow to throw off the onion flavor before she has produced the milk, (3) preventing the cow from getting onions, and (4) eradicating the onions from the pasture. C.J.B.

**116. New Phosphatase Test Checks Efficiency of Milk Pasteurization.** L. H. BURGWALD, Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Agr. Exp. Sta. Weekly Press Bul. No. 22, 36, Nov. 11, 1937.

The phosphatase test of Kay and Graham is depicted as a boon to the public health officials and control laboratories of commercial dairies. It is pointed out that the test can determine discrepancies of 1.0° F. in heating temperatures and variations as little as 5 minutes in holding time. Also, as little as 0.1 per cent of raw milk added to properly pasteurized milk can be detected by this test. W.E.K.

**117. Farmers Can Gain By Understanding Milk Market Plans.** Dept. of Rural Economics, Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Agr. Exp. Sta. Weekly Press Bul. No. 22, 40, Dec. 9, 1937.

Studies in Akron and Stark County markets show that many farmers market their excess over base milk through channels outside the market pool. A warning is issued urging farmers to understand fully the base allotment selling plan in order to avoid reduction in their base allotments. W.E.K.

**118. Some Unusual Flavors in Milk.** W. D. DOTERRER, Bowman Dairy Co., Chicago, Ill. Milk Dealer 27, 1, p. 54, Oct., 1937.

The author briefly discusses some of the unusual flavors in milk, such as chloro phenol, oxidized, and those caused by bacterial action. C.J.B.

**119. Methods of Measuring the Bacteriological Efficiency of Milk Plants.** E. H. PARFITT, Purdue Agr. Exp. Sta., Lafayette, Ind. Milk Dealer 27, 1, p. 58, Oct., 1937.

The author suggests several methods for measuring the bacteriological efficiency of a milk plant. These methods include those which determine

the ability of the plant operations to obtain as effective destruction of the organisms contained in the milk as secured under laboratory conditions, those methods which determine the extent of increase of count due to contaminated equipment, and those methods which can be used to determine thermophilic contamination. C.J.B.

**120. Summary of Present Legal Opinions of Milk Control Legislation.**

SAMUEL B. WEINSTEIN, Oregon Milk Control Board. *Milk Dealer* 26, 12, p. 43, Sept., 1937.

The author reviews court decisions regarding milk-control legislation from 1904 to the present time. C.J.B.

**121. Certified Milk as a Source of Vitamin C.** W. H. RIDDELL AND C. H.

WHITNAH, Kansas State College, Manhattan, Kansas. *Cert. Milk* 11, 126, p. 9, Oct., 1936.

These investigators point out that fresh milk is a more important source of vitamin C than it is generally assumed to be. The effects of ration, freshness, method of pasteurization and exposure to sunlight on the vitamin C content of milk are given. W.S.M.

Other abstracts of interest are numbers 73, 74, 75, 76, 83, 84, 85, 86, 90, 91, 92, 93, and 95.

## PHYSIOLOGY

**122. Lactation and Glycemia.** JULIO LORENZO Y DEAL, Director of the Hospital Pereyña Rossell, Montevideo, Uruguay. *Le Lait* 17, 162, p. 113, Feb., 1937.

The glycemia of the nursing mother varies so that the blood may show from 0.130 to 0.051 per cent sugar. No relationship appears to exist between the sugar content of the blood and the abundance of milk secretion, although higher milk yields coincide with blood sugar contents of 0.087 and 0.067 per cent. There appears to be no relation between glycemia and the age of the nursing infant. In the different phases of milking, following the glycemia at the beginning, middle, and the end of the milking it was found that in the cases of glycemia below normal, the diminution during milking was very slight, for glycemia above the normal, the milking had considerable influence on glucose-lactose metabolism and reduced the sugar content of the blood, and in cases where the sugar content of the blood was normal, both increases and decreases in blood sugar occurred during milking. A.H.J.

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International Association of Milk Dealers	The Royal Technical College, Copenhagen, Denmark
National Institute for Research in Dairying, Reading, England	United States Department of Agriculture
New York Association of Dairy and Milk Inspectors	

# ABSTRACTS OF LITERATURE

## BACTERIOLOGY

- 123. Standardization of Tablets for Determining Methylene Blue Reduction in Milk.** H. J. CONN, N. Y. Agr. Exp. Sta., Geneva, N. Y. *Am. J. Pub. Health* 27, 8, p. 793, 1937.

The variation in the dye content of Danish and American tablets is discussed. Methylene blue thiocyanate is preferable to methylene blue chloride. Methylene blue thiocyanate tablets are now being placed upon the market with the approval of the Committee on Standard Methods of Milk Analysis of the American Public Health Association. It is believed that American manufacturers will be able to put out a very uniform product.

M.W.Y.

- 124. Influence of Contaminating Bacteria on the Results of the Microscopic Test for Streptococci Mastitis.** C. S. BRYAN AND E. A. NELSON, Michigan Agr. Exp. Sta., East Lansing, Michigan. *Am. J. Pub. Health* 27, 9, p. 914, 1937.

Plating 0.1 cc. of a sterile 1-500 aqueous dilution of brilliant green into each tube prior to collecting a 10 cc. sample from the udder is recommended as a method of inhibiting contaminants.

M.W.Y.

- 125. Influence of Dead Bacteria on Microscopic Counts of Pasteurized Milk.** ARCHIBALD R. WARD AND CHARLES E. MYERS, Dairy Testing Lab., Detroit, Michigan. *Am. J. Pub. Health* 27, 9, p. 899, 1937.

The widely held belief that bacteria killed by pasteurization remain visible and are counted together with those remaining alive is not accepted by the author. Microscopic counts were made from milk held for varying lengths of time at 142° to 144° F. The author concludes that insufficient numbers of dead bacteria remain visible after pasteurization to impair the usefulness of direct microscopic counts made on pasteurized milk.

M.W.Y.

- 126. Modified Methylene Blue Reduction Technic.** H. R. THORNTON, Univ. of Alberta, Alberta, Canada. *Am. J. Pub. Health* 27, 8, p. 791, 1937.

Advantages and disadvantages of shaking the tubes of milk during incubation, the standard technic in Great Britain, are discussed. A comparison of the standard and modified tests on 332 market milks showed that neither test greatly excelled the other in accuracy, despite the greater variability

of the standard test. The author finds no justifications for adopting the modified test as standard on this continent. M.W.Y.

127. **The Need of Uniformity of Conditions for Counting Plates with a Suggestion for a Standard Colony Counter.** JACQUES ARCHAMBAULT, J. CUBOT, AND MAC H. MCCRADY, Div. of Lab., Quebec Ministry of Health, Montreal, Quebec. *Amer. J. Pub. Health* 27, 8, p. 809, 1937.

A counter for enumerating bacterial colonies on agar plates prepared from milk, water and other materials is described. The "Quebec Colony Counter" combines the essential features which makes for constant magnification and illumination, for the convenience and comfort of the operator, and for accuracy of counts. M.W.Y.

128. **The Method of Frost Modified by Van Oijen.** H. BARKWORTH. *Special Dairy Bact.*, College of Agr. of the Southeast, Wye, Kent. *Le Lait* 17, 168, p. 810, Sept.-Oct., 1937.

The modified method of Van Oijen was compared with the standard plate count procedure for determining the bacterial count of milk. The Van Oijen slides were incubated at 28° C. (82.4° F.) for 24 hours. The Van Oijen procedure was considered more precise than the plate count procedure for determining the bacterial count of milk. Details of the Van Oijen procedure are given. A.H.J.

129. **The Colon Bacilli Sensitive to Bacteriophage.** IRENE LIPSKA, Municipal Inst. of Hygiene, Warsaw, Poland. *Le Lait* 17, 169, p. 913, Nov., 1937.

Of the sensitive colon bacilli studied, 68.6 per cent of the strains had chemical properties characterizing them as fecal strains. Following the classification, *Bact. coli commune* had 31.4 per cent of sensitive strains. The greatest frequency of sensitive strains (100 per cent) was among the "birds and the marine invertebrates. The vigor of development at 22° C. (71.6° F.) and at 37° C. (98.6° F.) as well as the vitality of the strain as shown by the rate at which carbohydrates are fermented are inverse to the sensitivity of attack by bacteriophages. The sensitive strains of colon bacilli studied are characterized by a great constancy in their properties comprising more or less high resistance to bacteriophage. A.H.J.

## BUTTER

130. **Differences in the Lactic Acid Percentages in Butters.** E. O. WHITTIER AND C. S. TRIMBLE, Bureau of Dairy Industry, Washington, D. C. *Am. Creamery and Poultry Prod. Rev.* 81, 15, p. 518, Feb. 12, 1937.

Butter made from cream containing less than 0.25 acid and no starter will contain less than 0.025 apparent lactic acid; cream with 0.4 or greater per cent acid and no neutralizer will produce butter with 0.1 or greater per cent acid; sour cream neutralized to 0.2 per cent acid will contain 0.15 or more acid; cream treated with water or sweet skim milk or washed with alkaline water will show slightly decreased acid content; and storage of butter at somewhat higher temperatures than those usually used will show no increase in apparent acid content.

P.S.L.

**131. Progress in the Cream Improvement Movement.** J. O. CLARKE, Food and Drug Administration. *Am. Creamery and Poultry Prod. Rev.* 83, 15, p. 504, Feb. 10, 1937.

In 1935, 1936, and 1937 the per cents of cans of cream condemned were 4.00, 3.06, and 2.88 respectively. The program of improvement has just begun; better tests for measuring fitness of cream are needed. The sediment test does not measure decomposition; taste and odor standards are indefinite. Acidity, hydrogen-ion concentration and formol tests, and mold counts are being used to a greater degree. The first is used indirectly as an index of conditions of sanitation. The second may be impractical for creamery use since neutralizer nullifies the results. This is not true for the formol test. It is of value in judging relative degree of decomposition. A special test has been perfected for relative mold contamination and has given good results. Farm sanitation and frequent delivery of cream are objectives in any good cream improvement program.

P.S.L.

**132. Laboratory Manual of Methods of Analysis for the Butter Industry,** by the Research Committee (M. E. PARKER, *chairman*) of the American Butter Institute, 110 North Franklin St., Chicago, Ill.

This manual was designed to give the practical analytical procedures which may be used in a good creamery laboratory and which are used in the laboratory of the American Butter Institute. The official control methods are not included, for they are generally not adaptable to plant work.

The various chapters presented are on butter sampling; determination of yeasts and molds, moisture, fat, salt, and curd; pH; diacetyl and acetyl-methylcarbinol; sediment testing of milk, cream, and butter; titratable acidity; the Babcock test; keeping quality of butter; and checking acidity and salt test solutions.

The manual has been printed only on one side of the paper and it opens flat on the desk. The style is such as to be of greatest use in the creamery laboratory.

A.C.D.

**133. Delaying Oxidative Changes in Butter.** C. D. DAHLE AND D. V. JOSEPHSON, Dairy Dept., Penn. State College, State College, Pa. *Nat. Butter and Cheese J.* 28, 18, p. 18, Sept. 25, 1937.

A water extract of oat flour (Avenex No. 7) added to sweet or sour cream before pasteurization improved the keeping quality of the butter without imparting off flavor or incorporating sediment. The weight of Avenex used equaled 1 per cent of the fat. This amount seemed to give ample protection against oxidative deterioration of butter during storage for two months at 40 to 45° F. W.V.P.

134. **Butter Color and Vitamin A.** K. G. WECKEL, Univ. of Wisconsin, Madison, Wisconsin. *Nat. Butter and Cheese J.* 28, 17, p. 28, Sept. 10, 1937.

Natural yellow color of butter is due chiefly to carotene which in the animal body is transformed to vitamin A. A graph shows seasonal variations in carotene, vitamin A, and vitamin A activity in butter. Carotene accounts for 15 per cent of total vitamin A activity. There is little difference in total vitamin A activity of fat from four common breeds of cows when on the same ration, although carotene values vary widely. Feeds rich in carotene are listed and butter manufacturers are urged to encourage their use in order to improve nutritive value of butter. W.V.P.

135. **Stabilization of Butter Against Oxidized Flavor.** VIRGIL L. KOENIG, Okla. Agr. Exp. Sta., Stillwater, Oklahoma. *Nat. Butter and Cheese J.* 28, 15, p. 26, Aug. 10, 1937.

Butter prints wrapped in parchment paper which had been treated with oat flour (Avenized Parchment) and butter mixed with 0.06 per cent of a hexane extract of oat flour (Avenol) and then wrapped in ordinary parchment were stored at 0° F. and at 50° F. for seventeen weeks. Avenized parchment and Avenol showed definite protective action against tallowness and rancidity development. Avenol gave an off flavor to the butter during the first four weeks of storage. W.V.P.

136. **Improving the Keeping Quality of Butter with Treated Parchment.** C. D. DAHLE AND D. V. JOSEPHSON, Dairy Dept., Penn. State College, State College, Pa. *Nat. Butter and Cheese J.* 28, p. 6, July 25, 1937.

Prints of butter wrapped in Avenex-treated parchment kept better at 45° F. and and slightly better at -15° F. than butter wrapped in ordinary parchment. W.V.P.

137. **Keeping Down the Yeast and Mold Counts.** A. E. GROTH, Federal-State Butter Grader, Mankato, Minn. *Am. Creamery and Poultry Prod. Rev.* 83, 18, p. 702, March 10, 1937.

Mold spores may be kept down by keeping the creamery dry as with the use of overhead heating unit systems together with proper ventilation; by

frequent cleansing of the buttermilk and water storage tanks; keeping the covers of vats closed during pasteurization so as to pasteurize foam; drawing cream from gates of vats when heated and pouring into main body of cream; elimination of "dead pockets" in vats; checking seams and stuffing boxes frequently for contamination; thorough cleansing of pipe lines and pumps; especially thorough cleansing of the churn, for this is one of the chief sources of mold. Directions are given for care of the churn. P.S.L.

138. **Carotene in Butter Coloring.** R. B. STOLTZ AND T. S. SUTTON, Ohio State University, Columbus, Ohio. *Am. Creamery and Poultry Prod. Rev.* 84, 20, p. 614, Sept. 8, 1937.

The study concerns the use of semi-purified carotene in oil as a coloring material for winter butter, and as a method of increasing vitamin A content. Fifteen drops of Smaco carotene oil was added to a pound of butter and this was checked against a like sample containing no added carotene. Rats fed on the first sample showed a superior growth. Using a second group of rats,  $\frac{1}{2}$  pound of butter colored with another brand of carotene produced as much growth as a group fed untreated winter butter. It was found impractical to raise the vitamin A content of winter butter, however, to that of June butter. In the latter case, using Primatone, it was necessary to use four ounces per 100 pounds butterfat to get a desired color. P.S.L.

Other Abstracts of interest are numbers 148, 182, 201, 207, 208, 209, 215, 216, 217, 218, and 219.

## CHEESE

139. **Modifications in the Composition of Cheese in the Course of Prolonged Storage.** JEAN PIEN AND G. MAURICE, Lab. of the Farmers' Union, Paris, France. *Le Lait* 17, 170, p. 1040, Dec., 1937.

The moisture content of Camembert cheese diminished regularly from 53 to 15 per cent during 13 months storage. During this period a considerable loss in dry matter also took place as a result of fermentation. This loss can attain 15 per cent of the original dry matter in the cheese and can accordingly cause a considerable increase in percentage of fatty matter in the moisture-free cheese, as the fatty matter is not lost by fermentation. During storage of the cheese for 13 months, the soluble nitrogen attained a value of 41.5 per cent of the total nitrogen and ammonia nitrogen was 60 per cent of the soluble nitrogen. During the storage period the total nitrogen of the cheese decreased from 6.36 per cent to 5.58 per cent, the soluble nitrogen increased from 0.55 per cent to 2.20 per cent and the ammonia content from 0.17 per cent to 1.25 per cent. A considerable percentage of ammonia nitrogen is lost from the cheese at the same time that other volatile matter results from the processes of fermentation. The losses that have been

described occurred when the cheeses were stored at a temperature of 8 to 10° C. (46.4° to 50° F.).  
A.H.J.

140. **On the Change of the Lactose Content of the Curd after the Addition of Water in Making Edam Cheese.** H. A. SIRKS, Report of the Experimental Dairy Farm, Hoorn, Holland, pp. 159-174, 1937 (English summary).

The cheesemaker often adds water to the whey during the cheesemaking process to lower the lactose content of the curd. In this study it was found that at least 15 minutes must be allowed after the addition of the water before the whey is drained off the curd, to make certain that a lactose equilibrium has been reached between that in the curd and the whey. A.C.D.

141. **The Influence of Salt on the Composition and Quality of Brick Cheese.** E. L. BYERS AND W. V. PRICE, Univ. of Wisconsin, Madison, Wisconsin. *Nat. Butter and Cheese J.* 28, 14, p. 10, July 25, 1937.

A salt content of approximately 2 per cent by weight of the cheese seemed best. Less salt encouraged abnormal fermentation, high moisture, weak body and open texture. Excessive salting caused hard, harsh body, unnaturally white color, low moisture, loss of yield, delayed lactose fermentation and slow ripening.  
W.V.P.

142. **Identification of Roquefort Cheese.** IRA D. GARARD, ABRAHAM MINSKY, JAMES H. BAKER, AND VIOLA PASCALE, New Jersey College for Women, Rutgers Univ., New Brunswick, N. J., and Gar-Baker Lab., Inc., New York, N. Y. *Ind. and Eng. Chem.* 29, p. 1167, Oct., 1937.

The fraudulent substitution of cow's milk Blue cheese for the more expensive ewe's milk Roquefort cheese makes desirable an effective means of differentiation. The color of the fat and its Polenske value provide a reliable method. Analyses of the fat of numerous authentic Roquefort cheeses and of a wide variety of cow's milk cheeses are reported. Polenske values for sheep milk fat varied between 3.6 and 5.95, while those for cow's milk fat never exceeded 2.9. The color of the extracted fat from cow's milk cheese was always deep yellow; that from ewe's milk fat was always pale green. The diet of the animals was found not to be responsible for the difference in range of Polenske values. The Polenske number does not change with the age of Roquefort cheese.  
J.H.N.

143. **The Fermentation of the Egyptian Cheese "Mich."** EL GHERIANY MOSTAFA, Bact. of the Dairy Service in the Lab. of Vet. Path., Guizeh, Egypt. *Le Lait* 17, 168, p. 819, Sept.-Oct., 1937.

*Bacillus saccharobutyricus amylobacter* and *Streptococcus faecium* were regularly identified in "Mich." Often but not regularly "Mich" also contained *Bacillus mesentericus*, *Bacillus subtilis*, *Proteus ichthyosmus* and *Flavobacterium butyri*. On aging from 5 months to 14 months, the pH increased from 4.96 to 5.41, and the volatile acidity from 46 to 80 degrees Soxhlet. During the same period the butyric acid increased from 1.01 to 1.76 grams per 100 grams of the cheese. A.H.J.

Other Abstracts of interest are numbers 123, 126, 182, 207, 208, 209, 215, 216, 217, 218, and 219.

## CHEMISTRY

144. **The Physical and Chemical Properties of Casein Fat.** SAMUEL G. STEVENSON AND ALFRED L. BACHARCH, Glaxo Lab., Ltd., Greenford, Middlesex, England. *Biochem. J.* 31, 721, 1937.

It was reported in 1924 by other investigators that an anhydride of a hydroxystearic acid was obtained by repeated extraction of the residual fat occluded with casein separated from skimmilk.

The present authors investigated further the character of the fat extracted from lactic acid casein subsequently employed in preparing "fat-free caseinogen" used in Vitamin A-free basal diets. A comparison of the fat constants of the extracted fat with those for normal butterfat indicates that the casein fat is for the greater part indistinguishable from butterfat. A three-fold concentration of unsaponifiable matter in the casein following purely mechanical separation of most of the milk fat is of interest and may be of physiological significance. An absence of phosphorus and consequently phospholipins in the extract was noted. K.G.W.

145. **Metallic Content of Foods an Important Health Matter.** A. H. STAND, Consulting Chemist, New York City. *Cert. Milk* 11, 120, p. 7, April, 1936.

This article describes briefly spectrographic analysis as a new technique for a rapid qualitative and quantitative determination of metals in dairy products. By this method of analysis the author demonstrates the presence of tin in evaporated milk and the dissolving effect of cheese on tin foil.

W.S.M.

146. **The Value of the Volumetric Formol Procedure for Determining the Protein Content of Milk.** B. VAN DER BURG AND L. HABERS, Dairy Lab. of the Agr. Univ., Wageningen, Holland. *Le Lait* 17, 168, p. 805, Sept.-Oct., 1937.

The determination of the protein content of milk by the formol titration procedure was not found to have the required accuracy. A.H.J.

147. **The Determination of Carotene in Forage.** G. GENIN, Chemical Eng., Paris, France. *Le Lait*, 17, 169, p. 727, Nov., 1937.

The author discusses the modifications introduced by Peterson, Hughes and Freeman into Guilbert's method for determining carotene. A.H.J.

148. **The Determination of pH in the Service of Milk and Dairy Products Industry.** ALFRED KARSTEN. *Le Lait* 17, 169, p. 918, Nov., 1937.

Recently developed colorimetric and electrometric methods of determining the hydrogen ion concentration of dairy products are discussed. Photographs of the electrometric apparatus are shown. The significance of pH value in various branches of the dairy industry is discussed. Thus fresh milk outside the normal pH range 6.4 to 6.6 may indicate that it came from diseased cows. The use of pH control in the selection of milk for evaporated milk, milk powder as well as in the manufacture of butter and casein is discussed. A.H.J.

149. **Measuring Milk Film.** H. H. BECK, K. G. WECKEL, AND H. C. JACKSON, Univ. of Wis., Madison, Wisconsin. *Am. Creamery and Poultry Prod. Rev.* 83, 13, p. 444, Jan. 27, 1937.

Thickness of the film of milk passing over irradiation apparatus and its speed of travel are factors in the determination of the degree to which it is subjected to ultra violet light, and should be of value to designers of apparatus for irradiation. Thickness of milk film was measured by light. A narrow beam of light was thrown at an angle of incidence of  $45^\circ$  on the metal surface and the reflected beam measured. The reflected beam was again measured while a film of milk was run over the surface. The distance between the two parallel beams of reflected light was determined and from this the thickness of the film calculated by geometrical formula.

P.S.L.

150. **The Composition of Rabbit Milk Stimulated by the Lactogenic Hormone.** A. J. BERGMAN AND C. W. TURNER, Univ. of Missouri, Columbia, Mo. *J. Biol. Chem.* 120, 21, 1937.

The composition of rabbit milk obtained at various intervals after normal parturition was compared with the milk obtained from pseudo-pregnant rabbits in which lactation had been induced experimentally by the injection of lactogenic hormone for six days.

The amount of lactose and total solids in the experimental milk obtained from a rabbit having a 4+ rating (Gardner and Turner, in which the gland contains 0.76 to 1.0 per cent of lactose) were similar to that in the normal milk. The amount of fat was higher, and the ash content lower, in the experimental milk. K.G.W.

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

- 151. Observations on Concentrated Frozen Milk.** F. J. DOAN AND C. E. FEATHERMAN, Division of Dairy Mfgs., Penn. State College, State College, Pa. *Milk Dealer* 27, 2, p. 33, 62, Nov., 1937.

A report of studies on the concentration and freezing of milk as a means of preserving the product for a period of several weeks. The following factors were studied: (a) Degree of concentration, (b) homogenization, (c) length of holding period, (d) temperature of thawing.

The authors concluded that:

In concentrating and freezing milk for storage purposes a degree of concentration of approximately 3 to 1 appears to give most satisfactory results.

Homogenization prevents a rise of cream in the thawed, reconstituted milk. It decreases the tendency to churn, oiling-off and form cream plugs and also appears to aid in preventing tallowy flavor.

The length of time milk concentrated 3 to 1 can be held in a frozen condition and still produce a normal reconstituted product is somewhat indefinite and probably varies with the qualities of the original milk, the equipment used and the method of handling. From these results a storage period of 12 weeks seems quite possible.

The protein of concentrated frozen milk is apparently denatured by holding for long periods in the frozen state. This, however, appears to be a reversible action. In one sample encountered in these studies, protein which was apparently badly coagulated was re-dispersed in normal fashion by heating to temperatures between 150 and 160° F.

The thawing temperature does not exercise much influence on the properties of the reconstituted, concentrated frozen milk but a temperature of 120° F. appears to give less trouble in thawing and reconstituting than room temperatures of 50° F.

The number of trials made in this study and the number of samples included are too few to warrant very positive conclusions and it is hoped that the study may be continued in more detail, particularly with respect to the type of protein coagulation obtained when concentrated milk is held in the frozen state and with respect to the effect of concentration and freezing upon the curd tension.

C.J.B.

- 152. Chemists Produce "Wool" from Milk.** Home Economics Dept., Ohio Agr. Exp. Sta., Columbus, Ohio. *Ohio Agr. Exp. Sta. Weekly Press Bull.* No. 22, 44, Jan. 6, 1938.

A brief description of the Italian process is given and a prediction made that "wool" from milk may develop comparably to rayon.

W.E.K.

- 153. The Concentration and Properties of Vitamin H.** LELA H. BOOKER, Dept. of Chemistry, Columbia University, New York. *J. Biol. Chem.* 119, 223, 1937.

Methods for separating the relatively heat stable Vitamin H of the Vitamin B complex from low lactose whey powder, and from rice polishings is described. By the methods there is obtained 30 fold and 60 to 90 fold increase in concentration of the factor present in the whey powder and rice polishings, respectively. K.G.W.

- 154. Casein in the Fabrication of Plastic Materials.** G. GENIN, Paris, France. *Le Lait* 17, 168, p. 815, Sept.-Oct., 1937.

Casein precipitated by rennin is required for the making of casein plastics. A review is given of the methods for making casein plastics. Uses of casein plastics are discussed. A.H.J.

- 155. The Fabrication of Artificial Wool.** G. GENIN, Chemical Engineer, Paris, France. *Le Lait* 17, 169, p. 949, Nov., 1937.

A general discussion is given of the manufacture of casein and the fabrication of artificial wool fibers (Lanital) therefrom. Comparative carbon, hydrogen, oxygen, nitrogen and sulphur contents of true wool and Lanital are given. Except for the sulphur content which is higher in wool and the carbon content which is higher in Lanital, the composition of the two substances is much the same. In cold caustic soda Lanital dissolves more rapidly than wool. On burning wool and Lanital fibers, the residues are much the same. On boiling a sample of Lanital for 3 hours in an alkaline soap solution, it loses about 1 per cent of its weight, while on treating wool in the same manner, the loss in weight is about 10 times as great. Lanital is readily dyed. It is felted more readily than wool. A.H.J.

Other abstracts of interest are numbers 123, 124, 125, 126, 127, 132, 133, 134, 135, 136, 144, 145, 148, 149, 150, 157, 158, 159, 164, 165, 175, 176, 182, 188, 197, 199, 201, 202, 206, 207, 208, 209, 211, 212, 215, 216, 217, 218, and 219.

## DISEASES

- 156. Bovine Tuberculosis.** Editorial. *Am. J. Pub. Health*, 27, 9, p. 919, 1937.

This editorial describes an outbreak of tuberculosis in a Swedish rural community in which 50 persons, mostly children, suddenly showed signs of tuberculosis. Practically all had been supplied with raw milk from one dairy. One cow which had been passed as free from tuberculosis upon clinical examination by veterinarians had an acute tuberculosis of the udder which resembled the usual type due to streptococcus. However, the milk

teemed with tubercle bacilli. Clinical examination of milk cows, even by the most skillful veterinary surgeon, is not sufficient. "Not only should milch cows have the tuberculin test, but all market milk should be pasteurized, and this applies even to what we know in this country as Certified milk" states the editorial. M.W.Y.

- 157. A Milk-Borne Epidemic and Its Lesson.** Editorial. *Am. J. Pub. Health*, 27, 10, p. 1042, 1937.

Only pasteurization of all market milk can avert such tragedies as the outbreak of typhoid fever in England which is described. Approximately 718 persons contracted the disease, 31 cases being fatal. Pasteurization was immediately successful in bringing the outbreak to an end. M.W.Y.

- 158. Vaccination Against Bang's Disease in an Infected Dairy Herd with United States Bureau of Animal Industry Brucella Abortus Strain 19.** C. M. HARING, University of California, Berkeley, Calif. *J. Am. Vet. Med. Assn.*, N.S. 45-1, p. 52, Jan., 1938.

In a test of the resistance of dairy cattle to *Brucella* infection, 93 heifers were vaccinated with vaccine prepared from strain 19. During a four year period in which these heifers were in a dairy herd affected with brucellosis, this treatment apparently has been beneficial in retarding the spread of the disease. Strain 19 was not spread to healthy non-vaccinated cattle through association with vaccinated animals. Vaccine from strain 19 used on four cows induced a pronounced but temporary drop in milk production. Vaccination during advanced pregnancy of a cow kept in isolation brought about a typical brucellosis. J.W.W.

- 159. Consider Vaccines for Bang's Disease.** B. H. EDGINGTON, Ohio Agr. Exp. Sta., Animal Disease Control Laboratory, Reynoldsburg, O. *Ohio Agr. Exp. Sta., Weekly Press Bull.* 22, 45, Jan. 13, 1938.

Indiscriminate use of vaccines is not justified in any known scheme of Bang's disease control. Calfhood vaccination seems to have merit, although its use is still in the experimental stage. The use of vaccines appears most warranted in infected herds where removal of reactor cattle or adequate segregation of reactor and non-reactor animals is impractical. W.E.K.

## FEEDS AND FEEDING

- 160. Preparation and Nutritive Value of A. I. V. Alfalfa Silage for Dairy Cows.** MALCOLM BEESON, College of Agr., Madison, Wis. *Cert. Milk* 12, 137, p. 9, Sept., 1937.

The A. I. V. method of making silage is the preservation of green plant tissue by adjusting the pH to 3 or 4 with mineral acids.

The nitrogen distribution of A. I. V. alfalfa silage showed some proteolysis but only slight ammonia production. The carotene content of this silage was equal to that of the original green tissue.

The feeding of A. I. V. alfalfa silage to dairy cows definitely increased the carotene and vitamin A content of the milk. The blood also showed a much higher carotene content. No unusual changes in milk production resulted from feeding A. I. V. alfalfa silage. Rats fed mineralized A. I. V. silage showed a greater average growth than rats fed mineralized winter milk.

Blood and urine analyses indicated that the higher acid intake of the cows was being neutralized by means which prevented any deleterious effects on the animals.  
W.S.M.

161. **Molasses Silage Produces Unusual Results in Feeding.** C. B. BENDER, New Jersey Agr. Exp. Station, New Brunswick, N. J. *Cert. Milk* 12, 136, p. 9, Aug., 1937.

Feeding trials were conducted to study the color and flavor of milk produced by Guernsey cows when fed on various types of roughage. It was found that the color was maintained more uniformly in the groups receiving grass silage as part or all their roughage. The roughage ration which contained a high proportion of good quality molasses silage was superior to corn silage in its ability to produce milk of a high color and flavor.  
W.M.S.

162. **Need Vitamin A for Normal Reproduction.** T. S. SUTTON, Ohio Agr. Exp. Sta., Wooster, O. *Ohio Agr. Exp. Sta. Weekly Press Bull.* 22, 47, Jan. 27, 1938.

Recent experiments having shown that a ration deficient in vitamin A may produce permanent sterility in both males and females, it is recommended that the practice of feeding bulls low quality hay refused by other classes of livestock be discontinued.  
W.E.K.

163. **Making Silage from Hay Crops.** A. E. PERKINS, C. C. HAYDEN, C. F. MONROE, W. E. KRAUSS, AND R. G. WASHBURN, Ohio Agr. Exp. Sta., Wooster, O. *Ohio Agr. Exp. Sta. Bimonthly Bull.* 23, 190, pp. 3-12, Jan.-Feb., 1938.

Four methods of making legume silage (molasses, mineral acid, dry ice, and no treatment) are described briefly. Experiments with a special apparatus for exerting pressure on green material demonstrated that dry-matter content of the material to be ensiled is of prime importance. Appreciable loss of juice was obtained when green material containing less than 25 per cent dry matter was subjected to 8 pounds pressure per square inch. This corresponds to the pressure at the bottom of 26 to 28 feet of legume silage containing 30 per cent dry matter. Samples containing 30 per cent dry

matter lost no juice under 8 pounds pressure and little when under 12 pounds pressure. It is recommended that silo filling operations be so planned that the lighter, dried material go into the silo first and be followed with the heavier, wetter material.

W.E.K.

## FOOD VALUE OF DAIRY PRODUCTS

- 164. Vitamin D Milk—Its Rôle in the Prevention and Treatment of Rickets.** BENJAMIN KRAMER, Pediatric Research Lab., Jewish Hospital, Brooklyn, N. Y. *Cert. Milk* 11, 125, p. 3, Sept., 1936.

The author's conclusions in part are as follows:

The best evidence fails to reveal any difference in potency unit for unit between the different forms of vitamin D milk.

When milks containing a larger number of vitamin D units (400 to 430) are compared with those containing 135 units per quart, the former are found to be more effective as measured by rate of growth, calcium and inorganic phosphorus in the serum, calcium and phosphorus retention in the bones, and complete absence of roentgenological and clinical signs of rickets.

In the prevention of rickets in the prematurely born infant and in the treatment of the disease in the more resistant child, vitamin D milk must be supplemented by other antirachitic agents.

W.S.M.

- 165. Scientific Control of the Nutritional Content of Cow's Milk.** W. C. RUSSELL, Rutgers Univ., New Brunswick, N. Y. *Cert. Milk* 12, 132, p. 5, April, 1937; 12, 133, p. 5, May, 1937; 12, 134, p. 10, June, 1937.

This series of articles deals chiefly with a discussion on attempts which have been made to change the composition of milk by variation of the constituents of the ration. Thirty-four references on the more recent reports on this subject are given. The author concludes that generally speaking, the constituents of milk which give to milk its characteristic properties tend to remain constant in concentration, regardless of the composition of the ration. Thus, the milk proteins, fat, lactose, calcium and phosphorus, tend to be maintained at constant values, whereas vitamins A, C, and D, and iodine can be varied to a considerable extent by the level at which these substances occur in the ration.

W.S.M.

## HERD MANAGEMENT

- 166. Calf Raising Problems.** H. A. HERMAN, Missouri College of Agric. *Jersey Bulletin*, 57, 3, Jan. 19, 1938.

The maintenance of the dairy herd in numbers and established production levels requires the replacement of 20 to 30 per cent of the cattle annually. The necessity for this high replacement rate may be largely attributed to

Bang's disease, mastitis, sterility, and low production. Various reported studies show the average cow in the D.H.I.A. of this country remains in the herd from 3.6 to 4.7 years after freshening. The advantages and disadvantages of raising calves are discussed. It is suggested that in general the most satisfactory method of maintaining and improving the herd is by the raising of carefully selected calves. The calf crop should be culled thoroughly and only those from the best cows and sired by well bred sires kept. Calf mortality is exceedingly high in many herds, studies are reported showing that only about 50 to 75 per cent of all the heifers saved come into production. Scours, pneumonia, and general digestive disturbances account for most of the losses. A plan of careful management and strict sanitary measures is suggested as a means of combatting high calf mortality. The care of the newborn calf, and the raising of the calf to maturity by various feeding programs is discussed. The reader is referred to Mo. Agr. Exp. Sta. Bull. 377 for a description of the various methods successfully employed in calf raising. L.C.

**167. What Can be Learned from Production Records.** C. Y. CANNON, Iowa State College. Jersey Bulletin 57, 2, Jan. 12, 1938.

The objectives of cow testing programs are outlined and are stated to be: (1) to obtain a check whereby feeding and management improvements can be made on cows so as to increase financial returns, (2) to discover those superior cows whose blood should be used for the production of the next generation, and (3) to test the breeding powers and merit of bulls in service.

As butterfat production increases from 200 to 500 pounds, the increase in feed costs moves at the rate of about \$11 to \$12 per cwt. of butterfat. The value of the product increases more rapidly so that there is a greater difference between feed costs and value of product.

Records at the Iowa State College dairy farm shows that of every 100 two-year old heifers entering the herd, 77 per cent become three-year olds; 57 per cent become four-year olds; 41 per cent become five-year olds; 31 per cent become six-year olds; 22 per cent become seven-year olds; 14 per cent become eight-year olds; 11 per cent become nine-year olds. These figures vary but slightly from Iowa Cow Testing Association records. Such losses from the herd are caused by various agents. L.C.

**168. Water Your Cows Well.** C. C. HAYDEN, Ohio Agr. Exp. Sta., Wooster, O. Ohio Agr. Exp. Sta. Weekly Press Bull. 22, 44, Jan. 6, 1938.

Cows have a requirement for water from 4 to 5 times greater than the amount provided in their milk. Even dry cows need 10 gallons or more daily. W.E.K.

- 169. Pasture Improvement Gives Good Returns.** D. R. DODD, Ohio Agr. Exp. Sta., Wooster, O. Ohio Agr. Exp. Sta. Weekly Press Bull. 22, 47, Jan. 27, 1938.

In a series of tests extending over 7 years, a liberal treatment of 20 per cent superphosphate on limed pasture land produced 244 pounds of beef per acre in comparison with 135 pounds where no phosphate was used. When a high white clover content prevailed the difference was even greater. In terms of milk, the areas without phosphate produced the equivalent of 1078 pounds; all the phosphate areas, an average of 1950 pounds, and the high clover phosphated areas, 2255 pounds. W.E.K.

## ICE CREAM

- 170. Air Conditioning the Retail Ice Cream Outlet.** ARTHUR BEHRSTOCK, Walgreen Drug Co., Chicago, Ill. Ice Cream Rev. 21, 1, p. 22, Sept., 1937.

The experiences of a large drug company who have air conditioned 125 stores is given. The main reasons for air conditioning the stores are: (1) the comfort of the customers, (2) the health and, consequently, the temperament of employees, and (3) the greater cleanliness of merchandise. Best results are obtained when the temperature is not kept too low, and is raised as the outside temperature rises. Relative humidity is kept between 50 and 55 degrees. J.H.E.

- 171. Automatic Refrigerated Vending Machines.** Anonymous. Ice Cream Rev. 21, 2, p. 31, Sept., 1937.

Refrigerated ice cream vending machines have been developed. The machine described in the article has an ice bunker that holds 18 pounds of dry ice to refrigerate 102 ice cream novelties. The delivery operation is accomplished electrically. J.H.E.

- 172. Some Attractive Ice Cream Specialties.** JOHN CLAITOR, Birmingham, Ala. Ice Cream Rev. 21, 2, p. 44, Sept., 1937.

Directions are outlined for making ice cream specialties including fruit pie, baked Alaska, pumpkin pie, fruit cake, ice chocolate cake and nut roll. J.H.E.

- 173. Some Sanitary Aspects of Ice Cream Making.** M. J. PRUCHA, Dept. of Dairy Husb., Univ. of Ill., Urbana, Ill. Ice Cream Rev. 21, 2, p. 74, Sept., 1937.

In the summer of 1936, 480 samples of ice cream collected in different cities in Illinois had an average bacterial count of 1,000,000 per ml. Bacterial analysis of some 600 samples of unpasteurized ingredients added

to ice cream at freezing showed that these ingredients may be a real source of bacteria in ice cream. Other sources of bacteria in ice cream are reviewed. J.H.E.

174. **Manufacture of Sherbets and Ices.** S. L. TUCKEY, Dept. of Dairy Husb., Univ. of Ill., Urbana, Ill. *Ice Cream Rev.* 21, 3, p. 69, Oct., 1937.

The problems involved in the manufacture of ices and sherbets and their corrections are described. J.H.E.

175. **Serum Solids—The Rôle They Play in Ice Cream.** C. A. IVERSON, Dept. of Dairy Husb., Iowa State College, Ames, Iowa. *Ice Cream Rev.* 21, 2, p. 62, Sept., 1937.

Serum solids improve body and texture of ice cream but when used in excessive amounts pronounced defects occur which include, sandiness, soggi-ness, and poor melting quality. Serum solids may improve the flavor but as the milk solids are increased more flavoring must be added as the additional milk solids serve to subdue the flavoring. Serum solids may be sources of definite "off" flavors. J.H.E.

176. **Calculating an Ice Cream Mix Made in a Vacuum Pan.** HANS EDEL, Gehl's Guernsey Farms, Milwaukee, Wis. *Ice Cream Rev.* 21, 4, p. 34, Nov., 1937.

By use of a chart accompanying the article the author gives instruction for arriving at the quantity of various ingredients to be used in making a mix in the vacuum pan. J.H.E.

177. **Ice Cream Floor Plans.** Anonymous. *Ice Cream Trade J.* 33, 12, p. 8, Dec., 1937.

Floor plans for a 300,000 gallon ice cream plant showing arrangement of rooms and location of the various pieces of equipment is presented. A description of the plan of operation is also given. W.H.M.

178. **Common Flavor Defects in Ice Cream and How to Control Them.** W. C. COLE, Dairy Ind. Div., Univ. of Calif. *Ice Cream Trade J.* 33, 12, p. 16, Dec., 1937.

Off flavors in ice cream may be caused by (1) the use of inferior raw materials in the preparation of the mix, (2) the use of inferior flavoring materials in improper combination of flavors, and (3) the development of off flavors during storage. The use of pure vanilla extract rather than imitation vanilla and low storage temperature are recommended. W.H.M.

179. **1936 Ice Cream Production Figures.** Anonymous. *Ice Cream Trade J.* 33, 12, p. 40, Dec., 1937.

The production of ice cream and sherbet by each state and for all states during 1936 as reported by the U. S. Department of Agriculture is printed in tabular form. The total production of ice cream in 4,441 plants was 248,812,000 gallons representing an increase over 1935. W.H.M.

180. **The Association's Year.** ROBERT C. HIBBEN, Int. Assoc. Ice Cream Mfgs., Washington, D. C. Proc. 37th Ann. Conv. Int. Assoc. Ice Cream Mfgs. Vol. 1, p. 41, Oct., 1937.

Proposed legislation to be considered by the 1938 legislature and how it would affect the ice cream industry is discussed.

The information service of the association has been increased and numerous requests for publications have come from manufacturers, teachers of home economics and other interested groups.

The Ice Cream Merchandising Institute is disseminating regularly information helpful to distributors of ice cream as well as consumers. M.J.M.

181. **Our Industry.** MADISON H. LEWIS, Int. Assoc. Ice Cream Mfgs., The Borden Co., New York, N. Y. Proc. 37th Ann. Conv. Int. Assoc. Ice Cream Mfgs. Vol. 1, p. 24, Oct., 1937.

The three major activities of the International Association of Ice Cream Manufacturers at present are the accounting, merchandising, and legislative work.

The accounting bureau completed two general surveys, one an analysis of advertising and the other of sales. The analysis of sales showed a very definite shift in the past years. Bulk sales have decreased, package sales have increased, and there has been a sizable increase in novelty or specialty sales.

The Ice Cream Merchandising Institute has disseminated merchandising hints and materials to association members. Talking slide films have been prepared for retail to ice cream manufacturers for dealer and consumer education. The institute staff also held a series of fourteen merchandising meetings in different parts of the country.

Federal and State legislation which affects the ice cream industry is closely followed with the view of protecting the industry to the greatest extent possible.

Preliminary statistics indicate that the 1937 sales of ice cream will produce the greatest gallonage for any year in the history of the industry. M.J.M.

182. **The Dairy Industry's Future.** HARRY C. CALVERT, The Pfadler Co., Elyria, Ohio. Proc. 37th Ann. Conv. Int. Assoc. Ice Cream Mfgs. Vol. 1, p. 64, Oct., 1937.

A proposal is made that there be periodic meetings of representatives of the International Association of Ice Cream Manufacturers and Milk

Dealers, together with the Dairy and Ice Cream Machinery and Supplies Association for the discussion of all subjects of a controversial nature. Such discussions should lead to a closer alignment among the three groups in policies and programs affecting the dairy industries' national welfare. M.J.M.

- 183. Fundamentals of Official Ice Cream Control as We See Them in Birmingham, Ala.** L. C. BULMER, Jefferson County Board of Health, Birmingham, Ala. Proc. 37th Ann. Conv. Int. Assoc. Ice Cream Mfgs. Vol. 1, p. 66, Oct., 1937.

The fundamentals of ice cream control are: (1) complete pasteurization of all ice cream mix to a temperature of not less than 50° F. for a period of 30 minutes, followed by immediate cooling to the aging temperature; (2) positive control, so far as possible, of ice cream mix after pasteurization so that recontamination does not occur, and (3) improvement on esthetic grounds of the wholesomeness and quality of ingredients entering into ice cream.

The practice of shipping or transporting ice cream mix and freezing it at another place is viewed with considerable alarm by the author. M.J.M.

- 184. Report of Simplified Practice Committee for 1937.** RIDGWAY KENNEDY, JR., Chairman Simplified Practice Committee, Abbott's Dairies, Inc., Philadelphia, Pa. Proc. 37th Ann. Conv. Int. Assoc. Ice Cream Mfgs. Vol. 1, p. 88, Oct., 1937.

Sufficient study has been made by the committee to determine that ice cream can be kept in perfect eating condition for 12 to 48 hours in the ice compartment of the household refrigerator. The committee will meet with representatives of the National Electrical Manufacturers Association for the purpose of finding, first, how many types of household refrigerators are so constructed that the ice cube tray shelves are removable, so that ice cream in the present type of bulk and brick containers may be stored in it in quantities from a pint to several quarts. Secondly, the cooperation of manufacturers of household refrigerators will be secured, if possible, in so designing every model that the machines will accommodate the present bulk and factory filled packages.

An increasing number of ice cream manufacturers are adopting the "ice tray type" of package in order to make ice cream available to consumers in a form suitable for storage in household refrigerators of the type having space inadequate for present bulk and brick ice cream packages. M.J.M.

- 185. Europeans Eat More Ice Cream.** SAMUEL H. BAER, Blanke-Baer Extract and Preserving Co., St. Louis. Ice Cream Field 31, 8, p. 31, Dec., 1937.

Mr. Baer, who recently returned from an European trip, states that in 1923 almost 97 per cent of the ice cream sold in Great Britain was vanilla ice cream. Since that time the volume has increased many fold with an increased percentage of strawberry and ice creams other than vanilla, though vanilla ice cream still comprises over 75 per cent of their volume.

He states that improvement in the quality of ice cream has also taken place in England, especially due to controlled composition of the mixes used, but also because of the use of better flavors as well as improved ice cream machinery from the United States.

In 1930, the author states that there were practically no wholesale ice cream manufacturers in Paris, whereas now there are seven wholesale factories there producing between 50,000 gallons and 500,000 gallons annually. The most modern of these has American equipment. In Switzerland he states that there are now five large ice cream manufacturers. He reports the per capita consumption of ice cream as follows: United States over 3 gallons; Great Britain about one-half gallon; France about 1 pint; and Switzerland about one-half pint. W.C.C.

**186. Plea for Uniformity.** GLENN M. YOUNG, Missouri State Dept. of Health. *Ice Cream Field* 32, 1, p. 9, 1937.

The author states "all health departments, whether state or city, have the same objective—the health of the people. A great deal of confusion, unnecessary duplication and expense could be saved if there were greater uniformity in regulations governing the manufacture and sale of ice cream."

He states that due to the advent of the "counter freezer" ice cream plants present many problems to health officials that are sometimes different from those encountered in milk processing plants. Emphasis is placed upon the necessity of safeguarding the product after pasteurization, since "this is the last sanitary safeguard standing between the final product and the consumer." He continues, "Few public health officials who have the best interest of their people in mind would permit milk to be pasteurized at a plant and then be placed in cans and hauled to drug stores, restaurants, confectionaries or grocery stores for bottling. Exactly this is permitted with ice cream mix. It is even shipped long distances from one state to another. This in itself may not be so objectionable. It is how the mix is handled after reaching these places."

He outlined some of the commonly accepted practices regarding cleaning and care of equipment as a means of safeguarding the product. He points out, however, that, "one of the weakest links in the ice cream chain today is in retailing the product rather than in manufacturing it." W.C.C.

**187. Fall Season Suggests New Flavor Recipes.** JOHN CLAITOR. *Ice Cream Field* 31, 6, p. 47, Oct., 1937.

Suggested recipes for various flavored ice creams suitable for the fall are given. The list includes grape ice, grape meringue and the following ice creams: pumpkin, coconut, persimmon, pear, honeydew, melon, and avocado. W.C.C.

**188. Oat Flour as an Antioxidant.** SIDNEY MUSER, Musher Foundation, Inc. Ice Cream Field 31, 7, p. 31, Nov., 1937.

The author claims that "avenex" (an edible oatflour product) is effective in retarding the development of off flavors in dairy products. To substantiate this claim he refers to previously published results, reproducing some of them in table form. These results also indicate that when 0.5 per cent of "avenex" is used in ice cream that a reduction of at least 25 per cent of the stabilizer content, is desirable in order to avoid overstabilized ice cream. W.C.C.

**189. The Matter of Mix.** W. H. BROWN, Univ. of Illinois. Ice Cream Field 31, 3, p. 9, July, 1937; 4, p. 27, Aug., 1937.

The effects of adding various ingredients to ice cream mix after it has been pasteurized was considered in the light of sanitation and the bacterial content of ice cream. Special consideration was given pecan nut meats.

The author reviews some of the work previously published by other investigators to show that although the total number of organisms in the finished ice cream resulting from the addition of nut meats may be small, yet 50 per cent of the samples gave a positive test for *Bacterium coli*.

The author tried 69 different treatments of pecan nut meats but states that only 12 showed possibilities of being successful, and of these only 4 were recommended.

In a comparison of various methods of storing nut meats the author states "The best results from the standpoint of flavor and crispness were secured by storing in an open container at room temperature and the poorest results were secured by storing in an open container in the refrigerator room." These observations were made during the winter months.

Brief mention is also made of the preparation of peaches, bananas, and oranges for use in ice cream.

The following statements are taken from the conclusions drawn by the author:

1. The sanitary quality of pecan nut meats can be greatly improved by dipping in a 50-75 per cent boiling solution of sucrose plus 1 per cent salt followed by drying in a hot air oven. A marked improvement in the flavor of the nut meat usually resulted from such treatment.

2. The treated meats can best be stored in glassine bags at room temperature. The relative humidity should be around 42 to 50.

3. One-tenth of one per cent of sodium benzoate added to coloring materials will not inhibit the growth of bacteria.

4. The addition of 25 per cent alcohol to coloring materials will prevent the growth of bacteria and mold.

5. Coloring materials can be heated to 140°, 160°, or 180° F. for 30 minutes without injuring the quality of the colors. Additional heating, in some cases, slightly reduces the intensity of the color.

6. Strawberries and raspberries can be pasteurized to 145° F. for 30 minutes to improve their sanitary quality when used to flavor ice cream.

7. Peaches mixed with sugar can be boiled for 3 minutes without injuring the flavor of the fruit.

8. The flavor of oranges is not affected when dipped in 75-100 parts per million chlorine water to improve the bacteriological aspects of the fruit for use in ices or sherbets. W.C.C.

**190. Scoops as a Source of Contamination of Ice Cream in Retail Stores.**

ANDREW J. KROG AND DOROTHY S. DOUGHERTY, Health Dept., Plainfield, N. J. *Am. J. Pub. Health* 27, 10, p. 1007, 1937.

Bacterial counts of scoop samples were invariably higher than those of samples taken with a sterile spoon indicating surface contamination from scoops. The factors influencing the extent of contamination are discussed. Samples taken with dry scoops were lower than those taken with wet scoops. The authors conclude that if the ice cream scoops and other dispersing utensils are kept on a dry rack protected from flies, dust, and other sources of contamination, instead of in water, and rinsed with either hot or cold tap water after and before each use, the amount of contamination from the dispensing utensils can be greatly reduced. M.W.Y.

**191. Does Ice Cream Pay on Retail Dairy Routes?** FREDERICK E. JACOB, The Stevens-Davis Co. *Milk Dealer* 27, 2, p. 30, Nov., 1937.

The author discusses the pros and cons of ice cream on retail dairy routes. The following conclusions are drawn: If you have a high class product, high class trade with good home refrigeration, reasonably long summer climate, intelligent, aggressive routemen with some bonus incentive to increase their earnings, and not too much cheap package competition, then you should be selling ice cream on your retail routes. But—if you have numerous wholesale routes, less than ten or a dozen routes, too much union domination of routemen, routemen of the old "milk and cream only" school, too short summers, not enough finances to provide the proper refrigeration, a lack of advertising and merchandising facilities, you'd better look twice before you go into ice cream on retail routes. C.J.B.

**192. Walgreen's Trains Its Employees.** ARTHUR BEHRSTOCK, *Ice Cream Trade J.* 33, 8, p. 8, Aug., 1937.

A process, perfected over a period of years, has been developed by the Walgreen Company for training new recruits into a smoothly functioning

army of soda fountain employees. Prospective employees are judged on personal appearance, capacity to learn, and cleanliness. Once an application is approved the prospective employee is required to study a manual designed to help him learn the Walgreen way of doing things. A training course lasting from three days to a week, under the best man in the Walgreen service, is then given, followed by a trip through the commissary where he is impressed with the sanitary methods used in food preparation. An examination ends the employee's formal training. Bulletins telling of new methods are sent out and district meetings are held to discuss common problems.

W.H.M.

**193. Flavor Drives Build Sales 20 Per Cent.** DWIGHT ABBOTT. Ice Cream Trade J. 33, 8, p. 12, Aug., 1937.

Two weeks' concentration on a single flavor has produced better results than the advertising of many flavors of ice cream. Backbar dominations and colorful window displays have been used successfully by the Pangburn Company of Fort Worth, Texas, to produce increased ice cream sales during the past six months.

W.H.M.

**194. New Price Policies Pay Big Dividends in Sales.** J. EDW. TURFF. Ice Cream Trade J. 33, 8, p. 16, Aug., 1937.

The Hayden Ice Cream Company, Inglewood, California, has employed a volume dividend price plan to increase sales. Dealers are charged a base price and on the tenth of each month is paid an earned cash dividend based on volume of sales. The company has been able to maintain uniform retail price in its dealers' stores. New dealers are accepted only after a study of the area served by the store, and once the dealer is accepted the company assists him to get started properly by mailing invitations to his customers inviting them to the store for a free pint of ice cream. The dividend scale is based on actual differences in the cost of selling to the different dealers.

W.H.M.

**195. An Open Letter.** MALCOLM PARKS. Ice Cream Trade J. 33, 8, p. 20, Aug., 1937.

In a letter addressed to the ice cream manufacturer, a dealer calls attention to the many practices originated by the manufacturer which injured the dealer's business. Increased competition for retail stores and other outlets, cheap packages, and other items have only served to make it more difficult for the dealer to make a profit. The dealer states that he has decided to part company with the manufacturer and cooperate with other dealers in promoting their own manufacturing plant, and engage in other activities for the mutual benefit of the group.

W.H.M.

- 196. Refrigeration without Accidents.** EDW. R. GRANNIS, Industrial Engineer, Nat. Safety Council. Ice Cream Trade J. 33, 9, p. 17, Sept., 1937.

Most of the accidents which occur in refrigeration plants are preventable. Proper construction of the building to provide adequate light and ventilation, location of main service switch outside of compressor room, so that electrical current may be cut off in case of emergency, and the isolation of the boiler room from the compressor or generating room by an unpierced fire-resisting wall are recommended. Improper fusion welds on ammonia vessels are sometimes responsible for explosions. A device for the relief of excessively high pressures and the piping of safety valve outlets directly to the outside atmosphere are devised. Some automatic pressure limiting device to stop the action of the compressor at a pressure not higher than 90 per cent of the maximum allowable working pressure is advised. A simple way to detect an ammonia leak is with a stick wrapped in cotton and dipped into a small vial of muriatic acid. The saturated cotton is passed along the suspected pipe lines and fittings, and upon coming in contact with the smallest amount of escaping ammonia, a thick cloud forms above the stick. Gas masks should be available for use and attendants trained in the use of rescue apparatus. Careful operation of all refrigeration equipment by experienced men is of prime importance.

W.H.M.

- 197. Making the Mix in a Vacuum Pan.** P. S. LUCAS, Michigan State College, East Lansing, Mich. Ice Cream Trade J. 33, 9, p. 20, Sept., 1937.

"Advantages claimed for condensing the entire mix are several, such as the use of fresh milk and cream with consequent desirable effects on taste of the mix, not only through the use of fresher products but also by removal of off-flavors through heating in a vacuum." A pan in a combined market milk and ice cream plant offers a means of taking care of surplus milk. The amount of mix made and cost of raw materials are factors which determine whether the purchase of a pan is practicable or not. The cost of condensing the mix varies with capacity, in one plant with 150,000 gallons of mix it amounted to approximately 3 cents per gallon. Directions are given for calculating the amount of ingredients to use when making a mix in the pan.

W.H.M.

- 198. A Guide for Modernization.** Anonymous. Ice Cream Trade J. 33, 10, p. 6, Nov., 1937.

This article is devoted to a discussion of refrigeration equipment. Improvements made in evaporators, compressors, and condensers are described. Improvements in evaporators have been accomplished largely by increasing

the effectiveness of the surface. Modern evaporators are operated "flooded" and air circulation in the hardening room is used to speed up freezing and reduce frost accumulation. Two or more separate compressors provide a very flexible installation, and a booster compressor is a very effective method of handling low pressure vapor. Floor space can be conserved through the use of V-belt drives. Emphasis is placed on the desirability of designing a plant so that the highest possible suction pressure can be used, consistent with temperatures required.

A good condenser should have effective heat transfer surface and facilities for easy cleaning. Attention should be given the condensing water supply. The coldest water available consistent with cost should be used. Provision for removing non-condensable gas is necessary. An automatic purger connected to the top of the receiver is superior to hand purging and will usually pay in a short time by reducing power consumption. W.H.M.

199. Oat Flour as an Antioxidant in Ice Cream. W. S. MUELLER AND M. J. MACK, Mass. State College, Amherst. Ice cream Trade J. 33, 10, p. 24, Oct., 1937.

"The results secured in this experiment confirm previously published work which showed oat flour to have antioxidative properties when used in ice cream. The use of only 0.25 per cent of oat flour in the mix delayed the development of off-flavors during storage of the resultant ice cream, although 0.5 per cent proved more effective.

"Oat flour also has the properties of a stabilizer. The stabilizing action of the oat flour increased mix viscosity, improve the body and texture, and increased the melting resistance of ice cream. When oat flour is added to the mix, a reduction should be made in the amount of gelatin or other stabilizer used if an overstabilized condition is to be avoided. The results indicate that a reduction of at least 25 per cent of the gelatin content is desirable when 0.5 per cent oat flour is incorporated in the mix." W.H.M.

200. The Rôle of Eggs in Ice Cream. W. H. MARTIN, Kansas State College, Manhattan. Ice Cream Trade J. 33, 10, p. 29, Nov., 1937.

A review of the literature dealing with the use of eggs in ice cream is presented. Advantage gained from the use of eggs, kinds of eggs available, amounts and method of using eggs are some of the topics discussed. W.H.M.

Other abstracts of interest are numbers 125, 127, 132, 133, 134, 135, 136, 145, 148, 151, 157, 160, 164, 201, 202, 207, 208, 209, 212, 215, 216, 217, 218, and 219.

## MILK

201. The Lesser Known Constituents in Milk. G. GENIN, Paris, France. Le Lait 17, 168, p. 820, Sept.-Oct., 1937.

For 100 molecules of fatty acid combined in the form of triglycerides in the fatty matter of milk, there are 25 to 37 molecules of oleic acid, 24 to 27 molecules of palmitic acid, while butyric, myristic and stearic represent 7 to 10 molecules each. The other acids which are not very clearly defined consist of lauric, caproic, caprylic and capric. Milk fat also contains a small proportion, generally less than 1 per cent, of polyethenoidic acids of 20 or 22 carbon atoms in their molecule. The fact that the completely saturated acids are high in comparison with the contents of these acids in other natural fats has led the author to think that in milk fat the glycerides of the lower fatty acids are formed from oleoglycerides reformed by a combined phenomenon of oxidation and reduction. When cod liver oil is added to the feed of cows, certain of the polyrthnoidic acids characteristic of that oil, pass into the milk fat which then contains 5 to 7 per cent of these acids. At the same time the yield of fat in the milk is reduced and its composition is profoundly affected. The proportion of lower fatty acids is reduced to about one-fourth their normal value, the content of palymitic, stearic, and myristic acids is likewise reduced but the content of oleoglycerides is only slightly modified. These changes in the composition of the fat are only temporary and when cod liver oil is removed from the feed the fat composition becomes normal again.

Milk possesses the power to reduce methylene blue even if it contains none or few bacteria. Skimmilk does not reduce methylene blue as the reducing substance appears to have been removed by entrainment in the cream during separation. Holding the milk at 63° C. (145.4° F.) for 30 minutes destroys the enzymes or other substances responsible for the reduction of methylene blue. Raw cream diluted with water, reduced methylene blue. A number of substances were studied for the power to reduce methylene blue. Of the substances studied only aldehydes and hypoxanthine showed definite reducing activity to methylene blue. Attempts were made to separate the reducing substances from raw milk or cream by dialysis. Negative results were obtained but after dialysis certain of the enzymes in milk became inactive. Ascorbic acid was shown to play but an insignificant rôle in the anaerobic reduction of methylene blue.

The lipase activity in milk was determined in a buffer mixture at pH 8.5 using tributyrin as the substrate. The liberated butyric acid was removed by steam distillation and titrated. The lipase activity of milk showed considerable variation from one animal to another and from one stage of lactation to another. Lipase in milk is more readily destroyed than phosphatase. There is more lipase matter in the milk serum than in the fatty matter while the opposite is true of phosphatase. It is probable that there is no relation between the lipase and phosphatase activity of milk, the latter probably being associated with the efficiency of the mammary gland. Difficulties encountered in determining the catalase activity of milk are discussed and a review of applicable methods is given.

A.H.J.

- 202. The Effect of Light on the Vitamin C Content of Milk.** S. K. KON AND M. B. WATSON, Nat. Inst. for Res. in Dairying, Univ. of Reading, England. *Biochem. J.* 30, 2273, 1936.

The vitamin C content of milk was determined by the titration procedure using 2-6 dichlorophenolindo-phenol reagent. Evaluations were made before and after treatment with  $H_2S$ , permitting measurement of vitamin C as reduced ascorbic acid, and, reduced and reversibly oxidized forms (total ascorbic acid) respectively.

Milk giving a positive chemical test for vitamin C fails to reduce the indophenol reagent (reduced form of ascorbic acid absent) after exposure to 1 hour February sunshine while contained in glass. The reducing power can be partially restored by use of  $H_2S$  (oxidized form produced) but losses always take place.

The reaction shown above is due mainly to visible radiation of short wave length (blue and violet) although ultra violet radiation is also probably active. Visible radiation of longer wave lengths (yellow and red) are without effect.

When the dissolved oxygen of milk (in glass) is completely replaced by an inert gas, exposure to sunlight for 2 hours is without effect upon the reaction. Pasteurization had little effect on the influence of light on the ascorbic acid values of milk as determined by the titration procedure.

When milk is exposed to sunshine of sufficient intensity, the effect of light on vitamin C may be observed in two separate reactions: (1) a reversible change of the ascorbic acid to a substance (reduced into reversibly oxidized form) no longer able to bleach the indophenol reagent unless treated with hydrogen sulphide; to effect this change both light and oxygen are essential and this reaction does not proceed in the dark, and (2) a more gradual change of the product of the first reaction to a substance which cannot be caused by  $H_2S$  treatment to give a positive test with indophenol and which is biologically inactive. The latter product is suggested to be 2:3 diketo-1-gulonic acid.

A pint bottle of milk exposed to sunlight on the doorstep for half an hour and then kept for 1 hour in the dark loses fully half its original antiscorbutic properties. Pasteurization by the holder method does not affect the reduced form, but destroys the reversibly oxidized form, of ascorbic acid in milk.

K.G.W.

- 203. Cream Plug—What Can be Done to Avoid It.** JOSEPH BURNS, Capitol Dairy, Madison, Wisconsin. *Milk Dealer* 27, 3, p. 92, Dec., 1937.

The author discusses his experience with cream plug and draws the following conclusions:

To eliminate cream plug: Avoid excessive agitation. Heat and cool quickly to have short agitation time. Agitator should not go more than 70

r.p.m. **Avoid excessive heating.** Temperature in pasteurization of cream should not go above 150°. Avoid incubation temperatures. Cool cream immediately after separating if cream is to be held for any length of time prior to pasteurization. C.J.B.

- 204. The Use of Vending Machines in the Market Milk Industry.** JAMES R. HUDSON, Baker-Hubbel Dairy, Peoria, Ill. *Milk Dealer* 27, 3, p. 32, 48, Dec., 1937.

The author discusses the advantages and disadvantages of dispensing milk through vending machines in factories, schools, and office buildings. C.J.B.

- 205. New Type Conveyor Switches Effect Substantial Savings in Modernization Program.** *Milk Dealer* 27, 3, p. 30, Dec., 1937.

A description of how the installation of combiner and selector type divider switches in connection with bottle conveying installations effected a substantial saving in equipment, space requirement, and production costs at the Jausen Dairy, Hoboken, New Jersey. C.J.B.

- 206. Sale of "By Products" Growing Factor in Milk Industry.** *Milk Dealer* 27, 3, p. 29, Dec., 1937.

A survey by the *Milk Dealer* showed that almost 87 per cent of the dealers replying to a general questionnaire reported that they sold chocolate milk, 93 per cent advised that they handled buttermilk, 67 per cent sold orange juice, 80 per cent sold cottage cheese, 67 per cent sold butter, and 45 per cent handled eggs.

Chocolate milk accounted for 2½ per cent, buttermilk for 5 per cent, orange drinks for 3.8 per cent, and tomato juice for 0.7 per cent of the total volume of sales of those dealers reached by the questionnaire. The sale of tomato juice decreased while buttermilk and cottage cheese sales increased during the past two years. C.J.B.

- 207. The Structure and Composition of Foods, Vol. 3, Milk, Butter, Cheese, Ice Cream, Eggs, Meat, Meat Extracts, Gelatin, Animal Fats, Poultry, Fish, Shellfish.** ANDREW L. WINTON AND KATE BARBER WINTON. Published by John Wiley and Sons, 1937, pp. 524, Price \$8.00.

This book is the third of a series of four books dealing with the structure and composition of foods. The first 209 pages are devoted to the structure and composition of milk and milk products, the subjects covered in this review.

Apparently the authors have endeavored to compile the important contributions in the literature showing the composition of milk and milk prod-

ucts from the viewpoint of the analytical chemist, and to present them in an organized manner to give a systematic treatise of the subject. The many references cited are given as footnotes on each page. There has been little effort made to give a complete statement regarding each phase of the chemistry of milk and milk products but rather to quote the literature. The book has its chief value as a literature review and the reader should be able to interpret certain statements and expand some of the text to secure a true impression of the facts. For example, the paragraph on "microscopic characters" of human milk states that the fat globules in human milk are smaller than those in cow's milk but fails to specify their size except that they are less than  $10\ \mu$ . In the section on the fat globules in cow's milk their size is not definitely stated but data presented show them to vary "from less than 1 to over  $20\ \mu$ , the average, according to L. L. Van Slyke, being over  $2.5\ \mu$ ."

This book will be of value primarily as a handy reference to secure some of the important publications pertaining to a special aspect of the composition of milk and milk products. A.C.D.

**208. The Reliability of Flavor Judgments, with Special Reference to the Oxidized Flavor of Milk.** G. MALCOLM TROUT AND PAUL F. SHARP, Cornell Univ. Agr. Exp. Sta., Ithaca, N. Y., Memoir 24, June, 1937.

A total of 1207 ten-sample series, representing 12,070 taste judgments, of sodium-chloride, sucrose, lactose, lactic-acid, and quinine-sulfate solutions were judged in these studies. Judgment was made also on 3687 additional samples of sodium-chloride and sucrose solutions.

The temperatures at which the maximum discriminatory ability for the respective solutions was found, were as follows: sodium-chloride,  $21^{\circ}\text{C}$ .; sucrose,  $35^{\circ}$ ; lactose,  $35^{\circ}$ ; lactic-acid,  $21^{\circ}$ ; quinine-sulfate,  $21^{\circ}$ .

The sense of taste was found to be capable of discriminating as low as 1 per cent changes in concentration of the sodium-chloride solutions ranging in concentration from 0.13 to 0.20 per cent. With the sodium-chloride, sucrose, lactose, lactic-acid, and quinine-sulfate solutions, 10 per cent changes in concentration were readily detected.

The amount of substance required to produce a noticeable change in sensation was found to be but a very small percentage of the usually stated value.

The amount of retasting necessary before arriving at final judgment depended upon the concentration, its range, and the number of samples within the series. In ten-sample series of weak solutions, tasting some samples as many as ten times was found necessary.

The mean time, based upon approximately 250 trials each, required for four judges to place a ten-sample series of various solutions, was 4.4, 4.4, 4.4,

and 5.3 minutes, respectively. The mean time in placing the 1057 series was 5.04 minutes. No correlation was found between the time naturally required to place the series, and the accuracy of the judges.

After having placed a ten-sample series in order of concentration ten times, the judges had the samples sufficiently in mind to enable them to select any sample from the shuffled series and by taste alone name its concentration with remarkable accuracy. Graphs are presented showing the percentage distribution of these single-sample judgments of individual samples of several series by four judges. The mean differences between the true concentration and the estimated concentration, for solutions of sodium chloride selected at random from ten-sample series, were 0.029, 0.028, 0.019, and 0.042 per cent, respectively, for the four judges. The mean difference for 1987 individual samples tested was 0.029 per cent.

Retasting forty-sample series of sodium-chloride solutions five times improved the correlation from approximately 0.84 to 0.99. The final arrangement of a series was accomplished through end selection.

Samples of milk totaling 2152, involving 8608 taste judgments, were studied for the oxidized flavor.

The consistency of six commercial-milk judges in re-scoring milk was determined. The comparison between the percentage of all samples scored within a narrow range of scores, and the percentage of all samples re-scored with no deviation, seemed to give a more accurate indication of the consistency of scoring than did the percentage of identically re-scored samples alone.

E.S.G.

**209. The Handling of Milk and Milk Products.** A. T. R. MATTICK, Nat. Inst. for Res. in Dairying, Univ. of Reading, Ministry of Agriculture and Fisheries. Bul. 31, pp. 101, London, England. Obtainable from British Library of Information, 270 Madison Avenue, New York, Price 65 cents.

The fifth edition of this bulletin devotes considerable space to clean milk production and in addition gives an account of certain hitherto unpublished work carried out at the National Institute of Research in Dairying.

The essentials of clean milk production are presented in a clear and concise manner. Consideration is given to the cleanliness of farm workers, care of udder, method of milking and washing and sterilization of utensils and apparatus. The experimental data given relative to sterilization of equipment show the general superiority of proper steam sterilization over the use of disinfectants.

The hitherto unpublished research carried out at the Institute and presented in this bulletin includes sections on pasteurization, new media for the examination of milk, defects and off flavors in dairy products and the nutritive value of milk. Consideration is given to a comparison of the

nutritive value of raw, pasteurized and sterilized milk, the effect of pasteurization on vitamins and the effect of pasture and winter feeding on vitamins.  
J.C.H.

210. **Observe Changes in Ohio Milk Markets.** R. W. SHERMAN, Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Agr. Exp. Sta. Weekly Press Bull. 22, 50, Feb. 17, 1938.

During the past 10 years there has been a definite upward trend in butterfat content of milk delivered by 2500 shippers in four Ohio fluid milk markets—Canton, Cincinnati, Columbus, and Dayton. In Columbus and Dayton the average sales per day per dairy has increased. This is thought to indicate a reduction in the number of small producers. W.E.K.

211. **Production of Vitamin D Milk Increasing.** W. E. KRAUSS, Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Agr. Exp. Sta. Weekly Press Bull. 22, 9, Feb. 10, 1938.

It is estimated that about 3 per cent (400,000,000 quarts) of the fluid milk sold in the United States during 1937 was vitamin D milk. In 5 years the production of vitamin D milk has been comparable to that of pasteurized milk production during the first 5 years following its inception. W.E.K.

212. **Pasteurization in England and America.** Editorial. Am. J. Pub. Health 27, 9, p. 920, 1937.

In England, pasteurization has not received as much recognition as in this country although strongly advocated by leaders in the British medical and public health professions. Figures are given which indicate that market milk in large cities is much safer than in rural sections. Recent investigations at the National Institute for Research in Dairying at Reading, in Great Britain, have confirmed the fact that pasteurization has no appreciable effect upon the excellent nutritive qualities of milk. M.W.Y.

213. **Fermented Beverages from Milk.** ANTONIN MOULIN. Le Lait 17, 169, p. 946, Nov., 1937.

Details are given for the making of Kefir, Yoghourt, and Koumiss.

A.H.J.

214. **More Ammonia in Sour than in Fresh Milk.** A. E. PERKINS, Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Agr. Exp. Sta. Weekly Press Bull. 22, 45, Jan. 13, 1938.

Using an improved method developed by the author, it was found that whereas fresh milk contains only 3 to 5 parts of ammonia per million, sour milk may contain 20 or more times this quantity. This is probably due to the action of bacteria on the proteins of the milk. The amount of ammonia

in milk was not greatly changed by pasteurization or by storing and handling under proper conditions. W.E.K.

Other abstracts of interest are numbers 123, 124, 125, 126, 127, 128, 132, 134, 135, 144, 145, 148, 149, 151, 153, 156, 157, 158, 159, 160, 164, 165, 182, 183, 188, 196, 199, 215, 216, 217, 218, and 219.

## MISCELLANEOUS

**215. Minimum Wage Legislation.** BERNARD SUMMER, Member of the New York Bar. *Ice Cream Trade J.* 33, 12, p. 36, Dec., 1937.

A table is printed to show the powers of the Wage and Hour Commissions which have been established in various states adopting such legislation. In future articles, employee information, powers of the wage board, and violation and penalties will be tabulated. W.H.M.

**216. The Employer's Job.** WHITING WILLIAMS, Cleveland, Ohio. *Proc. 37th Ann. Conv. Int. Assoc. Ice Cream Mfgs.* Vol. 1, p. 29, Oct., 1937.

This is a discussion of employer-employee relationships. Some problems of the employee are set forth, an understanding of which is desirable for co-operative relationships between employer and employee. M.J.M.

**217. What's Around the Corner.** GUSTAVUS W. DYER, Vanderbilt Univ., Nashville, Tenn. *Proc. 37th Ann. Conv. Int. Assoc. Ice Cream Mfgs.* Vol. 1, p. 51, Oct., 1937.

The speaker presented his views of the functions of business and government. M.J.M.

**218. Our Economic Interdependence.** H. W. SUMNERS, Member of Congress, Dallas, Texas. *Proc. 37th Ann. Conv. Int. Assoc. Ice Cream Mfgs.* Vol. 1, p. 80, Oct., 1937.

The principal ideas expressed in this address are that the success of our government is based on the help and cooperation of the citizens of the country and likewise, success of any specific industry is largely dependent on the successful operation of industry in general. M.J.M.

**219. Present-Day Tax Problems.** C. A. JAY, Vice-Pres., Industrial, Commercial and Agricultural Conference, Dallas, Texas. *Proc. 37th Ann. Conv. Int. Assoc. Ice Cream Mfgs.*, Vol. 1, p. 72, Oct., 1937.

The present tax situation is set forth and it is urged that tax payers give more attention to the broad problem of taxation and public expenditures. M.J.M.

## PHYSIOLOGY

- 220. Increased Sodium Chloride Appetite in Pregnant Rats.** BRUNO BARELARE, JR., AND CURT P. RICHTER, Henry Phipps Psychiatric Clinic, Johns Hopkins Hospital, Baltimore. *Am. J. Physiol.* **121**, 1, p. 185, Jan., 1938.

The sodium chloride appetite of twelve female rats during pregnancy was studied by the method of self-selection. On the average, animals ingested over twice as much sodium chloride solution (3 per cent) in the ten-day period after conception as in the ten-day period before conception, and ingested over three times as much in the second half of the period of gestation. The sodium chloride intake in the first ten days postpartum fell back to a level only slightly above the pregestational level. It has been reported that during pregnancy there is a diminution in both anion and cation content of the blood. Among the cations, the sodium content suffers the greatest reduction, whereas among the anions the serum bicarbonate, serum protein, and organic acid are reduced. It appears highly probable that the animal in some way reacts to this reduction in electrolytes by voluntarily ingesting more sodium chloride.

Other changes in appetite probably occur during pregnancy. Further experiments are now being conducted with regard to this point. D.L.E.

- 221. The Experimental Production of Severe Homogeneous Osteoporosis by Gastrectomy in Puppies.** R. A. BUSSABARGER, SMITH FREEMAN AND A. C. IVY, Dept. of Physiology and Pharmacology, Northwestern University Medical School. *Am. J. Physiol.* **121**, 1, p. 137, Jan., 1938.

The stomach is essential for the normal growth and development of the bony skeleton of puppies. No evidence of rickets was observed in any of the roentgenograms made of these animals. The serum calcium, phosphorus, and phosphatase of the gastrectomized puppies were usually within the normal range. The deficient ossification of the bones is apparently due to a combination of three factors, namely, (a) the absence of hydrochloric acid which normally renders the less soluble calcium salts more soluble and assists in the maintenance of an acid reaction in the intestine; (b) the absence of the reservoir function of the stomach which increases the speed of intestinal transport of food substances; and (c) the presence of an "acid tide" after eating which tends to decrease calcium retention.

Although soluble calcium salts can be absorbed, two possible etiological factors remain. One is that food calcium is inadequately absorbed. The other is that after the calcium is absorbed, it is inadequately retained. In the absence of hydrochloric acid, organic acids such as carbonic and lactic acid and possibly the bile acids, assist in the absorption of calcium. Gastrec-

tomized dogs also manifest a hyperplasia of the bone marrow, which may contribute to the production of osteoporosis. D.L.E.

- 222. A Study of Protein Anabolism and Catabolism on a Nitrogen-Free Diet.** WALTER H. SEEGER, Samuel S. Fels Research Institute, Antioch College, Yellow Spring, Ohio. *Am. J. Physiol.* 121, 1, p. 231, Jan., 1938.

When rats are given a nitrogen-free diet during pregnancy the N for the young is transferred to them from the maternal reserves. If there is any wastage of N in this transfer, as a result of the young selecting a different assortment of amino acids than is liberated from the maternal reserves, it should appear in the urine. The newly formed tissue of the young will also have a catabolic phase of metabolism yielding N. The combined effect of wastage due to transfer of amino acids and that due to the catabolism of the young was measured and found to be small.

Pregnant animals have a much higher urine N coefficient than non-pregnant control animals, which suggests a higher metabolic rate. After the animals had given birth to young and had used a large quantity of their reserve protein they were continued on the diet until they died. At death the animals had lost 59.08 per cent of their original body weight and 58.42 per cent of their original N supply. As much as 64.4 per cent of the normal protein supply of the animal can be used before it succumbs, and the loss in body weight is of the same order of magnitude. D.L.E.

- 223. Studies on the Pancreas and Liver of Normal and of Zinc-Fed Cats.** D. A. SCOTT AND A. M. FISHER, Connaught Laboratories, University of Toronto, Toronto, Canada. *Amer. J. Physiol.* 121, 1, p. 253, Jan., 1938.

The authors mention that previous work has shown the presence of zinc in commercial preparations of insulin. More recently it has been demonstrated that the presence of minute quantities of zinc in suspensions of protamine and insulin not only increases the stability of these preparations but also prolongs their blood-sugar lowering effect. Although zinc is a necessary constituent of the diet for the normal growth of mice and rats and is present in all tissues and organs of the body in amounts almost as great as that of iron, its physiological function has not as yet been clearly demonstrated.

In trials conducted, cats fed on a high zinc diet (.25-.30 gram zinc oxide per day) for from 12 to 16 weeks showed a loss in weight averaging about 900 grams per cat. The amount of zinc per gram of pancreas and of liver was about 7 and 15 times, respectively, as great as that found in similar organs in the control group of cats. The total insulin content of the pancreas was not disturbed. Marked fibrotic changes in the pancreas of all the cats on a high zinc diet were observed. D.L.E.

- 224. The Adrenals and Gonads of Rats Following Thyroidectomy Considered in Relation to Pituitary Histology.** ISOLDE T. ZECKWER, Dept. of Pathology, School of Medicine, University of Pennsylvania, Philadelphia. *Am. J. Physiol.* 121, 1, p. 224, Jan., 1938.

As a result of thyroidectomy in young rats, there is retardation in kidney growth and increased weight of the pituitary in both sexes. In males there is no significant change in absolute weights of adrenals and testes, but the ratios of adrenals to kidneys, and the ratios of testes to kidneys are significantly increased. In females there is a decrease in absolute weights of the adrenals and gonads but this is proportional to retardation in kidney growth, so that the ratio of adrenals to kidneys and ovaries to kidneys are not significantly altered. Acidophiles and thyroidectomy cells can possibly be excluded as elaborators of adrenotropic hormone. By a process of exclusion, it is concluded that adrenal growth may depend upon a basophile cell.

D.L.E.

# JOURNAL OF DAIRY SCIENCE

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VOLUME XXI

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NUMBER 5

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## PROGRAM

### THIRTY-THIRD ANNUAL MEETING THE AMERICAN DAIRY SCIENCE ASSOCIATION

THE OHIO STATE UNIVERSITY  
COLUMBUS, OHIO

JUNE 14-15-16, 1938

AND

OHIO AGRICULTURAL EXPERIMENT STATION  
WOOSTER, OHIO

JUNE 17, 1938

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## PROGRAM COMMITTEE

C. J. BABCOCK, United States Department of Agriculture  
E. N. SHULTZ, American Guernsey Cattle Club  
W. E. KRAUSS, Ohio Agricultural Experiment Station  
H. P. DAVIS, (Advisory Member), University of Nebraska  
S. I. BECHDEL, (Advisory Member), Pennsylvania State College  
T. S. SUTTON, (Chairman), Ohio State University

## AMERICAN DAIRY SCIENCE ASSOCIATION

*The Thirty-third Annual Meeting*

Columbus, Ohio, June 14-16, 1938

AND

Wooster, Ohio, June 17, 1938

## GENERAL PROGRAM

*Monday, June 13*

- 1 P. M.-4 P. M. General Registration and Room Registration, Pomerene Hall. Dormitory rooms will not be available until Tues., June 14. Hotel and other rooms available June 13.
- 5 P. M. Visitors on the Ohio State Campus are cordially invited to attend the Convocation Exercises of the University, Ohio Stadium.

*Tuesday, June 14*

- 8 A. M.-9 P. M. General Registration and Room Registration, Pomerene Hall.
- 10 A. M.-12 NOON Opening Session, Campbell Hall Auditorium, 200.  
H. W. Gregory, presiding  
Address of Welcome.  
George W. Rightmire, President, Ohio State University.  
Response and Address.  
H. W. Gregory, President, American Dairy Science Association  
Address.
- 1 P. M.-2:30 P. M. General Session (Education), Campbell Hall Auditorium, 200.
- (1) The New Education, Dean Arthur J. Klein (by Invitation) College of Education, Ohio State University.
  - (2) Measuring the Results of Instruction in the Dairy Sciences. R. W. Tyler (by Invitation), Bureau of Educational Research, Ohio State University.
  - (3) Summer Practicum in Dairy Husbandry. A. A. Borland, Pennsylvania State College.
  - (4) A Service Course in Dairying for Home Economics Students. K. M. Renner, Texas Technological College.
- 2:30 P. M.-4:30 P. M. Manufacturing Section, Dairy Laboratories, Townshend Hall.  
Judging Conference for Coaches and Instructors only, L. H. Burgwald and J. H. Erb in charge.
- 2:30 P. M.-4:30 P. M. Production and Extension Sections Combined, Campbell Hall Auditorium, 200.

## Symposium on Nutrition.

4:30 P. M. Meeting of the Board of Directors, Campbell Hall, 203.

## SECTIONAL COMMITTEE MEETINGS

4:30 P. M. Testing Committee of the Extension Section and Breeds Relations Committee of the Production Section (Combined Meeting). Horticulture and Forestry Bldg., 208. C. M. Shepardson and Floyd Johnson (Chairmen).

## MANUFACTURING SECTION

Chemical Methods for the Analysis of Milk and Dairy Products, Campbell Hall, 216. L. C. Thomsen (Chairman).

Methods for the Bacteriological Analysis of Milk and Dairy Products, Campbell Hall, 217. H. Macy (Chairman).

Committee on Quality Program, Campbell Hall, 218. W. H. E. Reid (Chairman).

Committee on Feasibility of Establishment and Maintaining a Loose-leaf Manual of Laboratory Methods, Campbell Hall, 215. H. Macy (Chairman).

Committee on the Judging of Dairy Products. Campbell Hall, 204. G. Malcolm Trout (Chairman).

Committee on Methods of Determining the Curd Tension of Milk, Campbell Hall, 102. L. H. Burgwald (Chairman).

Committee on Score Cards for Sanitary Inspection of Dairy Farms and Milk Plants, Campbell Hall, 303. C. J. Babcock (Chairman).

Committee to Study Methods for Measuring the Oxidation of Milk Fat, Campbell Hall, 304. O. F. Garrett (Chairman).

## PRODUCTION SECTION

Committee on Rules for the Conduct of the Students' National Contest in Judging Dairy Cattle, Botany and Zoology Bldg., 110. I. W. Rupel (Chairman).

Committee on Awards for the Students' National Contest in Judging Dairy Cattle, Botany and Zoology Bldg., 208. A. A. Borland (Chairman).

Committee on Methods of Measuring Results of Pasture Investigations, Botany and Zoology Bldg., 209. R. H. Lush, (Chairman).

Committee on Standard Methods, Botany and Zoology Bldg., 100. A. E. Perkins, Chairman.

These same rooms will be available for a continuation of committee meetings from 8 to 9 A. M.

Wednesday morning. Committees wishing to schedule only one meeting can be called together at either the Tuesday Evening Hour or the Wednesday Morning Hour at the pleasure of the Committee Chairmen.

8:00 P. M. President's and Dean's Reception, Faculty Club, Administration Building.

*Wednesday, June 15*

8 A. M.—12 NOON General Registration and Room Registration, Pomerene Hall.

8 A. M.—9 A. M. Sectional Committee Meetings.  
Manufacturing Section Committees. Rooms as assigned for the previous evening.  
Production Section Committees. Rooms as assigned for the previous evening.

8 A. M.—9 A. M. Inspection of Extension Exhibits, Horticulture & Forestry, 203, 204, 205, E. C. Scheidenhelm, Chairman.

9 A. M.—11:30 A. M. Sectional Meetings.  
Production Section, Botany & Zoology Bldg., 100.  
Manufacturing Section, Campbell Hall Auditorium, 200.  
Extension Section, Horticulture & Forestry Bldg. 206.

11:30 A. M.—1 P. M. Complimentary Dairy Luncheon. Animal Husbandry Bldg.

1 P. M.—4 P. M. Sectional Meetings.  
Manufacturing Section, Campbell Hall Auditorium, 200.  
Production Section, Botany & Zoology Bldg., 100.  
Extension Section, Horticulture & Forestry Bldg. 206.

4 P. M.—5 P. M. Sectional Business Meetings.

8 P. M. Entertainment.

*Thursday, June 16*

8 A. M.—9 A. M. Committee Meetings.  
Production Section, Committee meeting rooms as previously scheduled.  
Manufacturing Section, Committee meeting rooms as previously scheduled.

9 A. M.—12 A. M. Sectional Meetings.  
Extension, Horticulture & Forestry Bldg., 206.  
Production, Botany & Zoology Bldg., 100.  
Manufacturing, Campbell Hall Auditorium, 200.

3:30—4:30 P. M. General Business Meeting, Campbell Hall Auditorium, 200.

6:30 P. M. Annual Banquet. Presentation of Borden Awards.

*Friday, June 17*

Wooster, Ohio

10:30–12 NOON

General Session, Earl Weaver, Presiding.

Welcome, Edmund Secrest, Director, Ohio Agricultural Experiment Station.

Response, Earl Weaver, Vice President, American Dairy Science Association.

## Papers:

- (1) The Contribution of Production Research to the Advancement of the Dairy Industry. H. B. Ellenberger, Head, Dept. of Animal and Dairy Husbandry, University of Vermont.
- (2) The Contribution of Manufacturing Research to the Advancement of the Dairy Industry. H. H. Sommer, Professor of Dairy Industry, University of Wisconsin.
- (3) The Interrelationships of Production and Manufacturing Research in the Development of the Dairy Industry. Ernest L. Anthony, Dean of Agriculture, Michigan State College.

12 NOON–1 P. M.

Box Luncheon.

1 P. M.

Tours to Points of Interest at the Ohio Agricultural Experiment Station.

## SECTION PROGRAMS

## EXTENSION AND PRODUCTION JOINT MEETING

W. E. KRAUSS, *Chairman*

Tuesday P. M., June 14, 2:30–4:30

Campbell Hall Auditorium, 200

*Symposium on Nutrition*

- P1—New facts in nutrition applied to dairy cattle. C. F. Huffman, Michigan State College.
- P2—Vitamin E and reproduction in herbivora. B. H. Thomas, C. Y. Cannon, S. H. McNutt, and G. Underbjerg, Iowa State College.
- P3—Relation of nutrition to the hormones. C. W. Turner, Missouri Agricultural Experiment Station.
- P4—Rumination and paunch activity. (Moving pictures of the “darkest place in the world.”) A. F. Schalk, Ohio State University.

## MANUFACTURING SECTION

Tuesday P. M., June 14, 2:30–4:30

Dairy Laboratory, Townshend Hall

Dairy Products Judging Conference for  
Coaches and Instructors

Professors L. H. Burgwald and J. H. Erb in charge.

## EXTENSION SECTION

E. N. SHULTZ, *Chairman*

Wednesday A. M., June 15, 9-11:30

Horticulture and Forestry Bldg., Room 206

*Testing Committee Report.* FLOYD JOHNSTON, *Chairman*

- E1—Methods of presenting different extension practices in the testing project. Glen W. Vergeront, University of Wisconsin.
- E2—Herd averages computed by the cow-year method versus herd averages based on cows on test at least 10 months. J. L. Lush and F. Johnston, Iowa State College.

FEEDING COMMITTEE REPORTS, K. L. TURK, *Chairman*

- E3—An extension program coordinating dairying, crops, farm engineering, farm management and forestry. A. R. Merrill, Connecticut State College.
- E4—A method used to illustrate the fact that higher producing cows make larger returns. W. T. Crandall, Cornell University.
- E5—An extension program in grassland farming. C. B. Bender, New Jersey Agricultural Experiment Station.
- E6—A method for preventing onion flavor in milk. C. E. Wylie, University of Tennessee.

## PRODUCTION SECTION

Wednesday Morning, June 15, 9-11:30

Botany and Zoology Bldg., 100

H. W. CAVE, *Chairman**Pasture, Hay and Silage*

- P5—Effect of fertilizer treatments on nutrients produced by pastures. R. A. Ackerman and H. O. Henderson, West Virginia Agricultural Experiment Station.
- P6—A method of studying the deficiencies of alfalfa hay and the feeding value of various feeds as supplements to alfalfa hay. C. F. Huffman, Michigan State College.
- P7—Air dried hay for dairy heifers. C. E. Wylie and S. A. Hinton, and J. W. Weaver, Jr., University of Tennessee and Tennessee Valley Authority.
- P8—The influence of certain rations and management practices on the rate of growth of Holstein Friesian heifers. R. G. McCarty and A. C. Ragsdale, University of Missouri.
- P9—The comparative nutritive value of sun cured pea vines, artificially dried pea vines and pea vine silage. J. C. Knott, R. E. Hodgson, and E. V. Ellington, State College of Washington and Bureau of Dairy Industry, U. S. D. A.
- P10—Experience in ensiling partially cured alfalfa, methods used, losses sustained, and feeding value. J. B. Shepherd and T. E. Woodward, Bureau of Dairy Industry, U. S. D. A.

- P11—Methods of making and feeding alfalfa molasses silage. B. R. Churchill and R. E. Horwood, Michigan State College.
- P12—The influence of the quality of protein in the concentrate mixture on the production of dairy cows fed mixed hay and corn silage. G. W. Salisbury and F. B. Morrison, Cornell University.
- P13—The influence of fineness of grinding on the coefficients of digestion on dairy cows. T. M. Olson and G. C. Wallis, South Dakota, State College.

#### MANUFACTURING SECTION

Wednesday Morning, 9–11:30

Campbell Hall, Room 200

C. J. BABCOCK, Presiding

##### *Market Milk*

- M1—Some factors affecting the estimation of fat in milk by the Babcock method. W. A. Caldwell and E. O. Herreid, Vermont Agricultural Experiment Station.
- M2—Cause and prevention of the decrease in fat test of composite samples. R. F. Holland, Cornell University.
- M3—A study of the Resazurin test as applied to cream. Herbert Jenkins, New England Dairies, Inc., Boston, Mass.
- M4—Studies of lipase action in milk. Vladimir N. Krukovsky and B. L. Herrington, Cornell University.
- M5—Observations on the lipase activity in cows' milk. J. C. Pfeffer, H. C. Jackson, K. G. Weckel, University of Wisconsin.
- M6—Detecting milk that may become oxidized. George R. Greenbank, Bureau of Dairy Industry, U. S. D. A.
- M7—The relation of oxidation–reduction potential to oxidized flavor in milk. George R. Greenbank, Bureau of Dairy Industry, U. S. D. A.
- M8—A study of the relation of titratable acidity to metal-developed oxidized flavor in milk. W. Carson Brown and R. B. Dustman, West Virginia Experiment Station.
- M9—Studies on the activated flavor of milk. J. C. Flake, H. C. Jackson and K. G. Weckel, University of Wisconsin.

#### EXTENSION SECTION

Wednesday P. M., June 15, 1:00–4:00

Horticulture and Forestry Bldg., Room 206

J. F. KENDRICK, *Chairman*

##### *Sire Committee Reports*

- E7—The use of electric fence in bull pen construction. J. W. Linn, Kansas State College. Discussion to be led by A. I. Mann, Connecticut Agriculture College.
- E8—Dairy cattle breeding schools. E. E. Heizer, Ohio State University.
- E9—Herd analysis and production pedigrees. S. J. Brownell, Cornell University.

J. C. NAGEOTTE, *Chairman*

*Calf Club Committee Reports*

- E10—Four H dairy programs, requirements and recommendations. H. A. Willman, Cornell University.  
 E11—Relationship between the dairy extension section and the national Four H rules committee. M. L. Flack, University of Nebraska.  
 E12—Use of movies in dairy extension instruction. J. C. Nageotte, Pennsylvania State College.

C. J. BABCOCK, *Chairman*

*Quality Committee Reports*

- E13—Report of Quality Committee Extension Section, American Dairy Science Association. C. J. Babcock, Chairman.  
 E14—Progress report of Quality Committee of Dairy Products of the Manufacturing Section of The American Dairy Science Association. W. H. E. Reid, Missouri Agricultural Experiment Station.  
 E15—Milk schools as a means of improving the milk supply. J. A. Nelson, Montana State College.

PRODUCTION SECTION

Wednesday P. M., June 15, 1:00—4:00

Botany and Zoology Bldg., Room 100

W. E. KRAUSS, *Chairman*

- P14—The relation of certain succulent roughages to the color and flavor of milk. H. H. Tucker, O. F. Garrett, C. B. Bender. New Jersey Agricultural Experiment Station.  
 P15—The effect of the level of feeding dairy cows upon the flavor of their milk. J. C. Hening and A. C. Dahlberg, New York Agricultural Experiment Station.  
 P16—A study of some of the physico-chemical effects of soybeans on the fat in cow's milk. R. W. Bratton, W. F. Epple, J. W. Wilbur, and J. H. Hilton, Purdue University.  
 P17—The vitamin D. content of the milk produced by Jersey and Holstein cattle receiving the same vitamin D. intake. G. C. Wallis, South Dakota State College.  
 P18—Plasma magnesium studies on the growing bovine. C. W. Duncan and C. F. Huffman, Michigan State College.  
 P19—The normal concentration of inorganic phosphorus in the blood of lactating dairy cows and factors affecting it. A. H. VanLandingham, H. O. Henderson, and G. A. Bowling, West Virginia Agricultural Experiment Station.  
 P20—The carotene content of market hays and corn silage as determined by a quantitative adsorption procedure. L. A. Shinn, H. G. Wiseman, E. A. Kane, and C. A. Cary, Bureau of Dairy Industry, U. S. D. A.  
 P21—Relationship between carotene, blindness due to constriction of the optic nerve, papillary edema and night blindness in calves. L. A. Moore, Michigan Agricultural Experiment Station.

- P22—The carotene requirements for normal reproduction. H. T. Converse and E. B. Meigs, Bureau of Dairy Industry, U. S. D. A.  
P23—Vitamin A for growth and reproduction in dairy heifers. I. R. Jones and J. R. Haag, Oregon State College.  
P24—Value of dried molasses and yeast for dairy calves. O. L. Lepard, P. E. Newman, and E. S. Savage, Cornell University.

## MANUFACTURING SECTION

Wednesday P. M., June 15, 1:00–4:00

Campbell Hall, Room 200

C. J. BABCOCK, Presiding

*Butter and Swiss Cheese*

- M10—Variation in the composition of milk and the effect on solids-not-fat. H. A. Herman, University of Missouri. (*Market Milk Con't*)  
M11—Studies on mold mycelia content of sour cream butter. J. Adams and E. H. Parfitt, Purdue University.  
M12—The effect of temperature upon score value and physical structure of butter. W. H. E. Reid and W. S. Arbuckle, University of Missouri.  
M13—Application of the Burri smear culture technic to the examination of butter. H. F. Long and B. W. Hammer, Iowa Agricultural Experiment Station.  
M14—The application of the phosphatase test to the butter industry. W. H. Brown and E. H. Parfitt, Purdue University.  
M15—Preliminary studies of the neutralization of cream for buttermaking. R. C. Townley and I. A. Gould, Michigan State College.  
M16—The relation of milk quality to grade of Swiss cheese. L. A. Rogers, Robert E. Hardell and Fred Feutz, Bureau of Dairy Industry, U. S. D. A., in cooperation with the Ohio State University and the University of Wisconsin.  
M17—Clarification of milk for the manufacture of Swiss cheese, with special reference to the use of mastitis milk. Kenneth J. Matheson, George P. Sanders, Lloyd A. Burkey, and J. Frank Cone, Bureau of Dairy Industry, U. S. D. A.  
M18—Control of types of organisms in high temperature starters. Dave Nusbaum and Walter V. Price, University of Wisconsin.  
M19—Methods of determining chlorine in milk and their application in the detection of mastitis. George P. Sanders, Bureau of Dairy Industry, U. S. D. A.  
M20—Controlling the fact content of Swiss cheese in southern Wisconsin. Walter V. Price, University of Wisconsin.

## EXTENSION SECTION

Thursday morning, June 16, 9:00–12:00

Horticulture and Forestry Bldg., Room 206

Sire Committee Reports, J. F. KENDRICK, *Chairman*

*Artificial Insemination*

- E16—Technique—Discussion by  
S. J. Brownell, Cornell University and  
E. J. Perry, New Jersey College of Agriculture
- E17—Use of artificial insemination in Farm Security Veterinary Associations. J. R. Allgyer, Farm Security Administration, U. S. D. A.
- E18—What we can learn from Denmark in the organized use of artificial insemination. E. J. Perry, New Jersey College of Agriculture.

## PRODUCTION SECTION

Thursday morning, June 16, 9:00–12:00

Botany and Zoology Bldg., Room 100

L. A. MAYNARD, *Chairman*

*Milk Secretion, Metabolism, and Udder Disease*

- P25—Initiation of lactation in the albino rat. R. P. Reece, New Jersey Agricultural Experiment Station.
- P26—Recent advances in our knowledge on the endocrine control of mammary development. E. T. Gomez and C. W. Turner, Missouri Agricultural Experiment Station.
- P27—The biological assay of "mammogen". A. A. Lewis and C. W. Turner, Missouri Agricultural Experiment Station.
- P28—Milk and fat production of dairy cows as influenced by thyroxine and anterior pituitary extracts. N. P. Ralston and H. A. Herman, Missouri Agricultural Experiment Station.
- P29—Vitamin C metabolism in the dairy cow. W. H. Riddell and C. H. Whitnah, Kansas Agricultural Experiment Station.
- P30—Fat metabolism of the mammary gland. J. C. Shaw and W. E. Petersen, University of Minnesota.
- P31—An enzymatic relationship to the synthesis of milk fat. Philip L. Kelly, University of Arkansas.
- P32—The effect of fasting and refeeding on milk secretion in the cow and goat. L. E. Washburn, University of Missouri.
- P33—The course of fasting energy production curves in the lactating and dry dairy cow under similar environmental conditions. L. E. Washburn, University of Missouri.
- P34—Nature of swelling in the cow's udder at calving time. W. W. Swett, C. A. Matthews, and R. R. Graves, Bureau of Dairy Industry, U. S. D. A.

## MANUFACTURING SECTION

Thursday morning, June 16, 9:00–12:00

Campbell Hall, Room 200

C. J. BABCOCK, *Presiding*

*By-products and Ice Cream*

- M21—Sodium per borate as a corrosion inhibitor for washing powders. Lawrence L. Little, Meadow Gold Milk Plant, Oklahoma City.

- M22—Sterilization by irradiation—a possible new tool for the dairy industry. O. F. Garrett and R. B. Arnold, New Jersey Agricultural Experiment Station.
- M23—Kefir Buttermilk. Lloyd A. Burkey, Bureau of Dairy Industry, U. S. D. A.
- M24—The present status of the development of fiber from casein. Earle O. Whittier, Bureau of Dairy Industry, U.S.D.A.
- M25—Whey solids in candy. Byron H. Webb, Bureau of Dairy Industry, U. S. D. A.
- M26—Effect of the cold storage temperature, pasteurization treatment, and homogenization pressure on the properties of frozen condensed milk. Raymond W. Bell, Bureau of Dairy Industry, U. S. D. A.
- M27—Consumer preference as related to the analysis of vanilla ice cream in Tennessee. Thos. B. Harrison, H. B. Henderson, and C. E. Wylie, University of Tennessee.
- M28—The use of moving pictures in ice cream investigations. W. H. E. Reid, W. S. Arbuckle and R. J. Drew, Missouri Agricultural Experiment Station.
- M29—Application of the phosphatase test to determine the efficiency of pasteurization of ice cream mix. A. J. Hahn and P. H. Tracy, University of Illinois.
- M30—Influence of certain mix components upon the rate at which freezing occurs in ice cream as measured by the dilatometer method. W. C. Cole and J. H. Boulware, University of California.

## PRODUCTION SECTION

Thursday P.M., June 16, 1:00-3:30

Botany and Zoology Bldg., Room 100

J. B. FITCH, *Chairman*

- P35—Some factors affecting the resistance of animals to mastitis. L. A. Burkey, E. B. Meigs, G. P. Sanders, and M. Rogosa, Bureau of Dairy Industry, U. S. D. A.
- P36—Preventing sudan grass poisoning. Frederick Boyd, O. S. Aamodt, G. Bohstedt and E. Truog, University of Wisconsin.
- P37—A report of the occurrence of four cases of Agnathia. Fordyce Ely, H. B. Morrison, and F. E. Hull, Kentucky Agricultural Experiment Station.
- P38—Maximum initial yield and persistency as inherited characters influencing total lactation yield. L. O. Gilmore, W. E. Petersen, and J. B. Fitch, University of Minnesota.
- P39—Herd averages computed by the cow-year method versus herd averages based only on cows on test at least 10 months. J. L. Lush and F. Johnston, Iowa State College.
- P40—Age and its influence on culling and life expectancy in dairy cows. D. M. Seath, Kansas State College, and J. L. Lush, Iowa State College.
- P41—The breeding efficiency of proved (aged) sires. J. R. Dawson, Bureau of Dairy Industry, U. S. D. A.
- P42—Twelve years with 1200 Holsteins. J. D. Brag, Ohio Department of Public Welfare.

- P43—Artificial insemination of dairy cattle. C. L. Cole, University of Minnesota.
- P44—Relation between rate of growth and milk and fat production. H. P. Davis and E. L. Willett, University of Nebraska.

## MANUFACTURING SECTION

Thursday P.M., June 16, 1:00–3:30

Campbell Hall, Room 200

C. J. BABCOCK, Presiding

*Cheese and Soft-Curd Milk*

- M31—A study of quality variations in summer and winter made cheeses. J. C. Marquardt, New York Agricultural Experiment Station.
- M32—Starters used in Wisconsin brick cheese factories. Willard L. Langhus, Paul R. Elliker, University of Wisconsin.
- M33—Methods which help to retain fat in American cheddar cheese at high temperatures. Harry L. Wilson, Bureau of Dairy Industry, U. S. D. A.
- M34—X-ray diffraction analysis of white specks in cheddar cheese. S. L. Tuckey, H. A. Ruehe and G. L. Clark, University of Illinois.
- M35—Studies on the ripening of blue cheese. C. B. Lane and B. W. Hammer, Iowa Agricultural Experiment Station.
- M36—Studies on the vitamin A content of cheese. I. L. Hathaway and H. P. Davis, University of Nebraska.
- M37—Plant experience with sonic soft curd milk. Leslie A. Chambers, Eldridge Reeves Johnson Foundation and University of Pennsylvania.
- M38—The digestibility of natural and processed soft curd milks. C. C. Flora and F. J. Doan, Pennsylvania State College.
- M39—The relationship between curd tension and curd size. Leslie A. Chambers and Irving J. Wolman, Eldridge Reeves Johnson Foundation and University of Pennsylvania.
- M40—Artificial gastric digestion of milk. Maurice E. Hull, M. & R. Dietetic Laboratories, Inc., Columbus, Ohio.

## PAPERS READ BY TITLE

*Production*

1. The relation of milking machines to the incidence of mastitis. Edward B. Meigs, Henry T. Converse, Division of Nutrition and Physiology, and Lloyd A. Burkey, Morrison Rogosa, and George P. Sanders, Bureau of Dairy Industry, U. S. Department of Agriculture.
2. Sudan grass hay vs. clover hay for dairy cows. C. E. Wylie and S. A. Hinton, University of Tennessee.
3. The extraction and assay of the hormones of cattle and sheep pituitaries. A. J. Bergman and C. W. Turner, University of Missouri.
4. Relation of lactic acid and glucose of the blood and glycogen in the mammary gland to milk secretion. W. E. Petersen, J. C. Shaw, University of Minnesota.
5. The carotene requirement of dairy calves. Ruel E. Ward, S. I. Bechdel, and N. B. Guerrant, Pennsylvania State College.

*Manufacturing*

6. Revised United States standards for quality of creamery butter. Roy C. Potts, United States Bureau of Agricultural Economics.
7. Effect of temperature and composition upon the physical properties and dipping qualities of ice cream. W. H. E. Reid, R. J. Drew and W. S. Arbuckle, University of Missouri.
8. A comparative study of metal and glass petri dish covers. Herbert Jenkins, New England Dairies.
9. Summary of experiment with the DeLaval standardizer. J. H. Frandsen, Massachusetts State College.
10. Methylene blue reduction time as an indication of the suitability of milk for the manufacturing of Swiss cheese. A. B. Erikson, C. A. Eckburg and E. Lee, The Borden Company.
11. Casein milk fat as a foam depressant in casein-clay slips. G. A. Richardson and N. P. Tarassuk, College of Agriculture, Davis, California.
12. The relationship of mastitis milk and soft-curd milk to the manufacture of Swiss cheese. Kenneth J. Matheson, Lloyd A. Burkey, George P. Sanders, and Robert R. Farrar, Bureau of Dairy Industry, U. S. D. A.

*Extension*

13. Texas one day shows. G. G. Gibson and E. R. Eudaly, Texas A. & M. College.
14. The Texas trench silo program E. R. Eudaly and G. G. Gibson, Texas A. & M. College.

**PROGRAM FOR LADIES' AND YOUNG PEOPLES'  
ENTERTAINMENT**

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**THIRTY-THIRD ANNUAL MEETING  
THE AMERICAN DAIRY SCIENCE  
ASSOCIATION**

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**THE OHIO STATE UNIVERSITY  
COLUMBUS, OHIO  
JUNE 14-15-16, 1938  
AND  
OHIO AGRICULTURAL EXPERIMENT STATION  
WOOSTER, OHIO  
JUNE 17, 1938**

**PROGRAM FOR LADIES' AND YOUNG PEOPLES'  
ENTERTAINMENT, AMERICAN DAIRY SCIENCE ASSOCIATION**

**COLUMBUS, OHIO, JUNE 14, 15, 16, 1938**

The following program has been arranged for the pleasure of the ladies and young people attending the convention. No program has been arranged for the ladies during the day on Tuesday, believing they would appreciate the time for rest and visiting. Headquarters will be in Pomerene Hall, women's recreational building at Ohio State University, located on the east side of Neil Avenue, adjacent to and south of Mirror Lake. The lounges will be available for use at all times. This will be a delightful place to meet your friends.

The young people's program starts at 1:30 P. M. Tuesday. Capable counsellors will supervise the activities of the various age groups. The Nursery School quarters in Campbell Hall, across Neil Avenue from Pomerene Hall, will be available for the care of pre-school children during the day. Experienced persons will be in charge.

Girls who have had experience in caring for children in the evening will be available for this service at a rate of 30 cents per hour with a maximum charge of \$1.00 per evening. Notice of such need should be filed with the registration clerks.

Pomerene Refectory, a delightful dining hall, will be open for meals each day. This is located on the first floor of Pomerene Hall. Service will be from 7:00 A. M. to 9:30 A. M., breakfast; lunch from 11:00 A. M. to 1:30 P. M.; evening from 5:00 P. M. to 7:30 P. M.

Mail will be received at the registration booth in Pomerene Hall and should be addressed in care of American Dairy Science Association, Pomerene Hall, Ohio State University, Columbus, Ohio.

**PROGRAM**

Subject to change dependent on weather and other unforeseen conditions. In such a case suitable announcements will be made and notices posted at registration booth.

*Tuesday, June 14, 1938*

- 1:30 P. M. The young people will meet at Pomerene Hall, Neil Avenue, Campus, for a tour of the University Campus.  
Ladies will have the afternoon free. Pomerene Hall lounges will be available.
- 7:30 P. M. Young people's party, Neil Hall, located one block south of University on the east side of Neil Avenue.
- 8:00 P. M. President's & Dean's Reception, Faculty Club, Administration Building.

*Wednesday, June 15, 1938*

- 9:00 A. M. Pre-school children will be cared for at Campbell Hall, located across Neil Avenue from Pomerene Hall.
- 9:30 A. M. Ladies will leave Pomerene Hall for a tour of the University flower gardens and green houses.  
Young people will meet at Pomerene Hall for outdoor games and contests arranged for young folk of all ages.
- 10:00 A. M. Ladies will see Mr. G. H. Poesch, floriculturist, give a demonstration on flower arrangement.
- 11:00 A. M. Ladies will visit the Ohio Archaeological and Historical Museum on the campus and will hear Mr. H. C. Shetrone, Director, discuss interesting facts regarding Ohio.
- NOON Lunch will be served at Pomerene Refectory in Pomerene Hall. The young people will have a private dining room where a desirable lunch will be served for 25 cents.
- 1:30 P. M. Motion pictures for the young folk at University Hall Auditorium.
- 2:00 P. M. Ladies will visit The Columbus Gallery of Fine Arts on East Broad Street. Mr. Philip R. Adams, director of the Art Gallery will conduct the tour. Transportation will be provided starting from Pomerene Hall promptly.
- 3:00 P. M. Swimming parties for the young folk. Arrangements will be made for the use of swimming pools on the campus. Competent persons will be in charge.
- 4:30 P. M. Tea for the ladies in the Lounge, Pomerene Hall.
- 8:00 P. M. Entertainment for all attending the convention. University Hall Auditorium and Natatorium. This will be an evening of fun and relaxation.

*Thursday, June 16, 1938*

- 9:30 A. M. Pre-school children will be cared for at Campbell Hall.  
Young people will meet at Pomerene Hall for a visit to the Ohio Archaeological and Historical Museum, University Campus.
- 10:00 A. M. Ladies will leave Pomerene Hall for a tour of the M. & R. Dietetic Laboratories and the Moores and Ross Milk Plant. The Dietetic Laboratories are famous for their baby food and other nutritional products while Moores and Ross are known to have one of the finest milk and ice cream plants. Transportation will be provided for the day.
- NOON The young people will lunch together at Pomerene Hall Refectory. Lunch will cost 25 cents.
- 1:00 P. M. Ladies will have a complimentary luncheon at the Columbus Country Club which is located on East Broad Street. This is one of the beauty spots of our city.
- 1:30 P. M. The young people will leave Pomerene Hall for a trip to the Columbus Zoo followed by a picnic in Riverside Park. They will return to the campus about 8:00 P. M.
- 6:30 P. M. Banquet at Neil House located between Broad and State Streets on High Street, across the street from the State Capitol.

**ABSTRACTS OF PAPERS PRESENTED AT  
ANNUAL MEETING  
GENERAL SESSION**

**2. Measuring the Results of Instruction in the Dairy Sciences. R. W. TYLER, Bureau of Educational Research, Ohio State University.**

Measuring the results of Dairy Science instruction is important not merely as a basis for assigning grades but to identify the difficulties students are having and to determine the effectiveness of particular courses, materials and methods of teaching. In order that measurement may serve these purposes more effectively it is necessary to develop better tests and examinations. Present examinations provide evidence regarding the information students are acquiring but the tests do not indicate how far students are achieving other important objectives such as the ability to apply facts and principles, the ability to observe accurately, scientific attitude, personality, characteristics and the like. To develop a more adequate measurement program it is necessary to formulate all of the important objectives of dairy science courses clearly in order to see just what things must be measured. After these objectives have been formulated and defined it is then necessary to devise means of testing for each of these important objectives. Experimentation in other science fields has shown the possibility of developing tests for many of these so-called "intangible" objectives of education and the use of these tests has made it possible to improve teaching at many points. Samples of such tests will be described in the paper and illustrations given about their uses.

**3. Summer Practicum in Dairy Husbandry. A. A. BORLAND, Pennsylvania State College.**

The commercial dairy world frequently asserts that college graduates have insufficient practical experience to justify their being placed in positions of even moderate responsibility. Undoubtedly this criticism has been made because in certain instances the facts have warranted the complaint. We all recognize that Colleges and Universities do not recommend their graduates in dairying as finished products, but do try to give their graduates a good fundametal education in chemistry, physics, mathematics, bacteriology and economics, with some training in dairying, so that with the later acquisition of experience they may become leaders in the dairy industry. Nevertheless, when a college graduate because of his lack of experience makes a poor showing in practical dairy work immediately after leaving college, considerable criticism of the institution from which he graduated is occasioned. It is to obviate this situation to as great an extent as possible that summer practicum work has been devised.

This subject, known at The Pennsylvania State College as D.H. 17, Summer Practicum, is required of all four-year students in Dairy Hus-

bandry. The subject carries 6 credits and calls for at least 6 weeks' practical work of not less than 48 hours a week in an approved dairy plant or on an approved dairy farm, depending upon whether the student is majoring in Dairy Manufacturing or Dairy Production.

The subject carries honor points if it is conducted under the immediate supervision of the instructor in the College Creamery or the College Dairy Barn in which case the student receives no financial remuneration. If taken at a farm or plant away from the college, the student may receive as much remuneration as his services warrant. In this case he gets credit for the subject provided his work has been satisfactory and he has turned in a creditable write-up of his summer's work, but receives no honor points.

The subject is taken during the vacation period following the junior year. Before June 1st the student must submit duplicate outlines concerning the information he expects to secure on the farm or in the plant during the summer. The instructor may make suggestions for the improvement of the outlines, then retains one for himself and returns the other to the student for his guidance. During the summer the student in addition to his practical work gathers data for writing up the dairy farm or plant during the first semester of his senior year. This write-up must be turned in during the first semester. The student is graded according to the merit of his paper and the report on his work by his employer.

This system insures that every student graduating from college has had at least six weeks of practical dairy plant or dairy farm work in addition to that which he received in college. The student when he graduates has more confidence in his own ability to do creditable work than when he has had no commercial experience. Frequently his summer work leads to permanent employment since students who have made good in their summer practicum work are in many cases offered positions by firms that would not otherwise employ additional help. Finally, students with some commercial experience are in so much better shape to do satisfactory practical work that a much higher percentage of them succeed in their chosen field of work than would otherwise be the case.

#### **4. A Service Course in Dairying for Home Economics Students. K. M. RENNER, Texas Technological College.**

A discussion of the content material for a course in dairying to be given to students in Home Economics. An outline of such a course, which has been given at the Texas Technological College for the past eight years, will be presented. The desirability of such a course has been well established and the results secured have been very satisfactory. The course is elected primarily by students fitting themselves to do County Home Demonstration work and by those preparing for teaching in rural communities. The course includes a rather wide variety of subject matter and the field of dairying with

which the type of student mentioned naturally comes in contact in his or her field work. Practical laboratory work is given the students, enabling them to become more familiar with the methods of carrying on dairy demonstration work with rural people. Three credit hours are allowed each student.

## PRODUCTION SECTION

### P1. New Facts in Nutrition Applied to Dairy Cattle. C. F. HUFFMAN, Michigan State College.

As the spread between the price of feed and butterfat narrows, the interest in more economical production of butterfat increases. This means a greater use of home grown feeds, especially roughage.

Greater use of pasture for dairy cows is receiving considerable attention. The classical work of several experiment stations during the past few years has demonstrated the high nutritive effects of grasses and legumes when used before lignification interferes with digestibility. The discovery of so-called "grass factor or factors" in milk produced during the summer has also enhanced interest in pasture. The importance of using fertilizers on pastures to improve yield of nutrients has been investigated.

The conservation of roughage for winter use is one of the most important problems in dairy cattle nutrition. Artificial drying of roughages yields a good product but the cost appears prohibitive.

Several experiment stations, here and abroad, have studied grass and legume silage. The use of mineral acids in sufficient amounts to reduce the pH of the silage mass below four, preserves the nutrients especially carotene, but the cost appears high for general use.

The use of molasses in making legume silage is enjoying considerable popularity. Recent work indicates that legumes are low in fermentable carbohydrates. The molasses furnishes additional fermentable carbohydrates for the production of lactic and acetic acids. There is a diversity of opinion regarding the value of molasses for this purpose, however. Some recent investigations indicate that good legume silage can be made without any additions, and that the exclusion of air is the main factor to be considered.

Some workers in the field of energy relations have concluded that the productive or net energy value of a feed depends on the balance of nutrients in that feed. The nutritive properties of individual feeds are not always additive when they are combined in the ration, because of the associative effects of feeds in digestion and their supplementing effects in metabolism. A well balanced ration should exert an energy value about in proportion to its total digestible nutrients.

Several workers have reported results with alfalfa hay as the sole ration, which indicates efficient use of total digestible nutrients. Other investigators have reported poor utilization of the total digestible nutrients on a ration of

alfalfa hay alone, which indicates a wide variation in the feeding value of alfalfa hay.

The essential amino acids necessary in the ration of milking cows remains unsolved. The evidence is increasing that cattle are able to use nitrogen in the form of urea and ammonium salts in place of protein. Rumen flora are believed to play a rôle in this phenomenon.

Cobalt has been added to the list of essential minerals. Recent work in various parts of the world indicates that a deficiency of this element occurs among cattle, which results in anemia. Nickel and copper appear to aid in cobalt utilization.

Iron, potassium and magnesium are required by cattle but there is no evidence that a deficiency of these minerals occurs under field conditions.

Fluorine, selenium and molybdenum at fairly low levels in the ration are detrimental to health. Pasture grasses high in manganese may disturb magnesium metabolism.

Recent work indicates that the addition of yeast to the ration of calves receiving a limited amount of whole milk improved growth. The vitamin G in the yeast was believed to be responsible for this observation.

The advisability of feeding cod liver oil to dairy calves under farm conditions has been further investigated. The results are not definite.

Recent investigations have failed to show that vitamin E is required by ruminants. The possible relation of vitamin K to sweet clover poisoning in cattle is of interest.

The relation of feed of the cow to flavor and to the nutritive value of milk has also received considerable attention during the past year.

The three "mystery diseases," namely: milk fever, grass tetany and ketosis, have also been studied from the standpoint of nutrition.

## **P2. Vitamin E and Reproduction in Herbivora. B. H. THOMAS, C. Y. CANNON, S. H. McNUTT AND G. UNDERBJERG, Iowa State College.**

Numerous scientific data reveal that qualitative inadequacies of the diet can evoke reproductive disorders in animals. Among the vitamins the one invariably thought of in this connection is vitamin E. The deleterious effect of avitaminosis E on fertility in rats and mice and on decreased hatchability of eggs has been demonstrated. Vitamin E has been recommended recently as an adjuvant to practical rations for improving the fertility and fecundity of farm animals. Whether or not avitaminosis E will interfere with the normal development of the fetuses of herbivora as in female rats has not been demonstrated heretofore.

To determine the importance of vitamin E to reproduction among farm animals we restricted goats, sheep and rabbits during one or more generations to a vitamin E-deficient ration. Essentially the same basal feed mixture of ground grains and their by-products and finely chopped alfalfa was fed to all

animals. The vitamin E occurring naturally in this mixture was destroyed or inactivated by treating it with an ether solution of ferric chloride and subsequently aging each treated batch of feed until it acquired a decidedly rancid odor. All animals received the basal ration, water and iodized salt, *ad libitum*. The treated basal ration was fortified at feeding time with a vitamin E-free supplement compounded primarily to protect against other possible avitaminoses.

Our study of this problem has demonstrated that the dietary vitamin E requirements of different species of animals may differ markedly. For example: weaning male rats restricted to the ferric chloride treated ration exhibited initial stages of testicular degeneration in two months and became permanently sterile in eight months or less. Female rats reared similarly repeatedly resorbed their young.

Unlike rats, the reproductive behavior of male and female goats apparently was unaffected during a period of four and one-half years, although restricted at all times to the same ferric chloride treated ration. Thus, our original flock of seven goats was expanded to forty-eight without exhibiting reproductive disorders attributable to avitaminosis E. Similarly, twelve original male and female rabbits kindled 137 fully developed young in two years, however not without an occasional reproductive disorder, the cause of which we have not yet ascertained. Whether or not the vitamin E requirements of sheep compare with those of rats or goats is impossible to state definitely at present. Our studies with sheep and rabbits are being expanded.

There is a dearth of scientific data evaluating the vitamin E requirements of farm animals. Yet statements have been made inferring that vitamin E therapy will markedly decrease reproductive disorders prevalent in herds, flocks and studs. Investigations have shown that vitamin E is present in most of the ingredients commonly used in compounding livestock and poultry rations. To be sure, there is no knowledge of the changes in vitamin E activity which may occur in milled feeds through processing or aging. However, it is evident that the vitamin E requirements of certain species of animals differ markedly from those of others. Obviously stockmen should be cautioned against relying on vitamin E therapy as a panacea for decreased fertility and fecundity until additional information is obtained.

### **P3. Relation of Nutrition to the Hormones. C. W. TURNER, Missouri Agricultural Experiment Station.\***

The relation of nutrition to the hormones which influence body growth, reproductive processes, and milk secretion is largely unexplored. The effect of improper amount or balance of the nutritive constituents of the ration has usually been interpreted as due to the absence of or deficiency in the essential

\* Contribution from the Department of Dairy Husbandry, Missouri Agr. Exp. Sta., Journal Series No. 552.

components (amino acids, energy, minerals, and vitamins) for the growth of the body or the synthesis of milk. Only recently has the rôle of nutrition in relation to the secretion of the various hormones affecting these processes begun to be appreciated.

Clearly, a deficiency of essential amino acids in the ration will influence the rate of growth or milk secretion. However, if the ration is deficient in constituents, either organic or inorganic, which influence the rate of secretion of one or more hormones concerned in the process of growth or milk secretion even though the ration were adequate in respect to the precursors of growth or milk, growth and milk secretion would be limited.

The need of certain trace elements in nutrition may be due to their presence in certain hormones. Thus the requirement of iodine is probably in very large part due to the need of the thyroid gland in the synthesis of thyroxine. As knowledge of the chemical composition of the hormones of the pituitary and other endocrine glands develops, the requirement of trace elements or other endocrine glands develops, the requirement of "trace elements or other essential groupings will be recognized.

The interrelation of the vitamins and the hormones will undoubtedly develop as this field develops. Already numerous studies have shown a dependence of the pituitary in the secretion of the gonadotropic hormone upon vitamin B. Without this vitamin, estrus cycles soon cease and reproduction is held in abeyance until this vitamin is added to the ration. The quality of protein appears to influence the secretion of the gonadotropic hormone and practical observations with cattle on deficient rations (quantity as well as quality) show delayed estrum until rations improve. One symptom of phosphorus deficiency is a prolonged dietary anestrus which is probably traceable to a deficiency in rate of secretion of the gonadotropic hormone.

**P5. Effect of Fertilizer Treatments on Nutrients Produced by Pastures.** R. A. ACKERMAN AND H. O. HENDERSON, West Virginia Agricultural Experiment Station.

Eight plots totalling 39 acres were seeded to blue grass, red top, and white clover in 1931. Three different fertilizer treatments with check, in which no fertilizer or lime was added, were duplicated. The fertilizer treatments at the time of seeding and later follow:

Check Plots	No lime or fertilizer applied.
P-L Plots	Limed to pH 6.5 in 1931.
	500# 20% superphosphate in 1931, and in 1933.
P-K-L Plots	P-L as above.
	100# Muriate of potash in 1931 and in 1933.
N-P-K-L Plots	P-K-L as above.
	200# Nitrate of soda per year except 1937, when only 100# was applied.

Pure bred Ayrshire heifers balanced as nearly as practicable into equal age and weight groups and receiving no supplemental nutrients have been grazed on these plots. By varying the number of heifers on a plot, all pastures have been grazed as uniformly as possible. Weights were taken on two consecutive days at the time the heifers were put on the pastures, at the time of their removal, and at intervals throughout the season.

The number of pasture days and gain in weight per acre, together with the average daily gain, was kept for the heifers on each plot. From this data the total digestible nutrients was calculated by using the figures given in the Morrison Feeding Standard. Over the five year period the complete fertilizer treatment more than doubled the number of pasture days and the number of pounds of total digestible nutrients produced on an acre, and the other treatments also showed a great increase in yield.

This is a partial progress report of five years (1933-37 incl.) results in a long time extensive pasture experiment conducted by the Department of Agronomy and Genetics, Department of Dairy Husbandry, and the Reymann Memorial Farms, of the West Virginia University.

**P6. A Method of Studying the Deficiencies of Alfalfa Hay and the Feeding Value of Various Feeds as Supplements to Alfalfa Hay. C. F. HUFFMAN, Michigan State College.**

The trend toward a greater use of home grown rations has prompted workers at several experiment stations to study the feeding value of alfalfa alone for milking cows. Experiments at Kansas and Oregon Stations showed that alfalfa alone did not yield as much milk energy as the total digestible nutrients in the alfalfa indicated. These results were in agreement with the energy value attributed to alfalfa hay by Fraps, Armsby and European workers. Graves and associates and workers at the Nevada Agricultural Experiment Station, however, have reported efficient utilization of the total digestible nutrients of alfalfa hay when fed alone.

In a long time alfalfa feeding experiment at the Michigan Agricultural Experiment Station cows receiving alfalfa supplemented with either corn, oats, barley or a complex grain mixture failed to maintain milk production and body weights during medium and low production, when they were getting most of their nutrients from alfalfa hay. The total digestible nutrients of the alfalfa exerted a low feeding value.

In reversal experiments either of short time nature or lactation reversals, the question of residual or carry over effect appears important. With our technique cows are placed on alfalfa alone at calving and left on this ration until the stored factors necessary to balance the alfalfa for milk production are exhausted. This is indicated by a sharp decline in milk flow. When milk produced had dropped to between 20-30 pounds daily a portion of the total digestible nutrients in alfalfa is replaced by an equal amount of total digestible nutrients in the feed to be tested.

The addition of soybean oil and corn oil to alfalfa alone did not affect milk production materially. Further evidence that fat was not the first deficiency of alfalfa alone was an increased milk yield when beet pulp or solvent soybean oil meal replaced a part of the alfalfa hay.

The addition of either corn, cottonseed meal or corn gluten meal in place of alfalfa has resulted in increased milk production.

This method appears to offer an effective, inexpensive method of studying the relative milk producing value of various supplements to alfalfa hay alone.

**P7. Air Dried Hay for Dairy Heifers.** C. E. WYLIE AND S. A. HINTON, University of Tennessee, and J. W. WEAVER, JR., Tennessee Valley Authority.

### *Hay Drying*

To "make hay while the sun shines" has never been very successful in "rainy seasons." Artificial drying may produce splendid hay, but large dehydration plants costing \$3,500 and up are of little use to the farmer who grows from 20 to 60 tons of hay a year.

The usual procedure in making hay, for one day, is to cut the hay in the morning, windrow it about midday, and put it in the barn in the afternoon if the weather has been favorable for curing. The principle of the special barn curing system as described in this report is to let the sun and wind of the open field do about 75 per cent of the curing the first day, and finish up the other 25 per cent after the hay is safely stored in the barn.

When the hay is 75 per cent cured it still has 35 to 45 per cent moisture—twice as much as is safe for storage, but just right for handling because the leaves are limp and there is very little loss from shattering. This damp hay is stored in the loft. The loft is equipped with a system of air ducts through which air from a blower is forced upward through the hay or drawn down through the hay, as desired. This process gradually removed the excess moisture and keeps the hay at about the wet-bulb temperature. By blowing air through the hay during the day only, and allowing the excess moisture in the hay to move to the surface of stems and leaves over night, curing is completed practically as soon as by continuous blowing. The use of a solar heat absorber to heat the air blown through the hay holds considerable promise as an economical means of speeding up the curing process with the same size blower.

Equipment for fitting up a hay loft of 20 to 30 tons capacity for their system can be bought and installed for about \$300.00, or less than 10 per cent of the cheapest dehydrator now available.

### *Feeding Trials*

During the winter of 1937-38 two groups of dairy heifers have been fed the hay which has been field cured and that which has been air cured.

Analyses have been made of the hay. An experiment is being run with rats to determine the vitamin A content. The growth of the heifers in each group was determined by weight, height, and girth measurements. At the completion of this trial the data indicate no significant difference in the feeding value of the hay cured by these two methods when fed to dairy heifers. Any difference, therefore, in these two methods of curing hay would seem to be in the amount and quality of the hay harvested from the field, including hay which has been damaged by rain.

**P8. The Influence of Certain Rations and Management Practices on the Rate of Growth of Holstein Friesian Heifers.** R. G. McCARTY AND A. C. RAGSDALE, University of Missouri.\*

The length of time and the amount of food nutrients required to grow dairy heifers to proper size and maturity for breeding are factors of considerable economic importance to dairy farmers. Obviously, any shortening of the growth period and consequent reduction in the unproductive life of the heifer may tend to reduce the cost of raising and will result in milk production and financial returns at an earlier age. This paper presents the results of an investigation on the influence of three rations on the rate of growth of Holstein heifers. The rations used were (1) milk and alfalfa hay supplemented only with common salt, (2) a basal ration herein also referred to as a "Rapid Growth" ration, and (3) the "Rapid Growth" ration modified to include 10 per cent of dehydrated cereal grasses.

Growth in all groups was measured by gain in live weight and height at withers and compared with "normal" as presented by Ragsdale in Mo. Agricultural Experiment Station Bulletin 336.

The heifers in Group 1 averaged 17.3 per cent *below* normal in weight and 4.1 per cent *below* normal in height at withers at six months of age. At 12 months the corresponding percentages below normal were 22.1 per cent and 5.0 per cent; at 18 months 11.8 per cent and 3.27 per cent; and at 24 months 13.8 per cent and 3.8 per cent, respectively.

The heifers in Group 2 averaged 23.2 per cent *above* normal in weight and 6.6 per cent above normal in height at withers when 6 months of age. At 12 months the corresponding percentages above normal were 32.6 per cent and 4.8 per cent; and at 18 months, 33.8 per cent and 5.9 per cent, respectively.

The corresponding data for the heifers in Group 3 in terms of percentages *above* normal were 20.3 per cent in weight and 5.8 per cent in height at withers; at 12 months 26.9 per cent and 4.7 per cent and at 15 months 27.5 per cent and 4.4 per cent, respectively. The heifers in all groups are being continued and data for later ages will be reported together with feed nutri-

\* Contribution from the Dairy Husbandry Department, Missouri Agr. Exp. Sta., Journal Series No. 560.

ents required for gains and certain other observations when the investigation is complete.

- P9. The Comparative Nutritive Value of Sun Cured Pea Vines, Artificially Dried Pea Vines and Pea Vine Silage.** J. C. KNOTT, R. E. HODGSON AND E. V. ELLINGTON, State College of Washington and Bureau Dairy Industry, U. S. D. A.

Pea vines remaining after the peas had been removed for canning were preserved for feeding purposes by field curing, artificially drying and by making them into silage. Digestion experiments were conducted with wether sheep to determine the nutritive value of the three types of forage. The results are based on six digestion trials, two for each kind of feed obtained in different years. On the basis of the composition of the dry matter and the apparent digestibility a high quality roughage feed may be produced from preserving pea vines in either of the three ways. The choice of methods used by farmers depends largely upon climatic conditions and equipment available.

- P10. Experience in Ensiling Partially Cured Alfalfa, Methods Used, Losses Sustained, and Feeding Value.** J. B. SHEPHERD AND T. E. WOODWARD, Bureau of Dairy Industry, U. S. Department of Agriculture.

During the period June 2 to 8, 1937, a concrete silo 14 feet in diameter was filled to a depth of over 37 feet with 115,325 pounds of partially cured, first cutting alfalfa. The object was to see if partially dried hay could be readily stored and preserved in the silo and to see how the feeding value compared with a quality of hay which with good luck might have been made from the crop. The dry matter content of the alfalfa ranged from 42 to 73 per cent, averaging 56 per cent. The alfalfa was finely chopped (cutter set for  $\frac{1}{4}$ -inch cut). No water or other material was added. Two large loads of freshly cut alfalfa with 70 per cent moisture were run in on top. The top was weighted down with 50 pounds of additional weight per square foot of surface area.

The silo was opened on October 20 and the silage fed from October 21, 1937, to March 18, 1938. The settled silage was 30 feet 4 inches deep.

The maximum temperature reached during the storage period 6 feet below the surface was 109.4° F.

Spoilage on top was only 7 inches deep except right at the edge where it extended down 2 to 5 feet, averaging 3 feet. The total weight of the top spoilage was 4,445 pounds. This 4,445 pounds of spoiled material is probably the equivalent of 6,350 pounds of the green alfalfa as ensiled. This 6,350 pounds is 5.5 per cent of the total amount of alfalfa ensiled.

Below the top spoilage the silage was of excellent quality and all edible except for (1) a thin coating of mold varying from about 1 inch in thickness

to a thin film next to the wall in the upper half of the silo, due to the roughness of the concrete, and (2) limited areas of moldy silage around several silo doors. The average loss in weight of 55 samples, one from each load, buried in the silo was 3.10 per cent, principally dry matter. Analyses showed that most of the dry matter lost was nitrogen-free extract.

The carotene content of the alfalfa averaged 63.6 parts per million of dry matter when put in, and 47.75 when removed. The pH of the silage ranged from 4.61 to 4.78.

Two feeding trials of 60 days each were conducted to compare the value of the alfalfa silage and U. S. No. 2 leafy alfalfa hay as the sole roughage. The cows were given all the roughage they would consume. There was some excess which was weighed back daily. Enough grain (equal parts corn, oats, and wheat bran) was fed to approximate the Haecker feeding standard.

Averaging both feeding trials: The cows getting alfalfa silage consumed 7.6 per cent more dry matter in their roughage than the cows getting alfalfa hay. The milk production of the cows getting alfalfa silage declined only 15.6 per cent in 60 days, while the milk production of cows getting alfalfa hay declined 24.7 per cent. The average gain in live weight per cow was 16 pounds in 60 days for cows getting alfalfa silage and 18 pounds for cows getting alfalfa hay.

**P11. Methods of Making and Feeding Alfalfa Molasses Silage.** B. R. CHURCHILL AND R. E. HORWOOD, Michigan State College.

The successful preservation of high quality roughages in the upper peninsula of Michigan has always been a major problem. The adoption of a heavy roughage feeding program together with two successive years of small grain crop failures has made the problem even more acute. Investigations are being conducted at the Upper Peninsula Experiment Station at Chatham with ensiling as a possible method of preservation. Twenty-five tons of alfalfa molasses silage were ensiled in 1935 and a like amount again in 1936, using 1.5 per cent molasses. Dairy feeding trials with these lots of silage indicated that from a feeding standpoint, a satisfactory alfalfa molasses silage could be made. The trials also indicated that the biggest problem was the method of making the silage.

Twelve lots of legume silage were ensiled in September, 1937, to determine if possible the most satisfactory method of ensiling. Lots varied from none to three per cent molasses and with sugar replacing the molasses. Lots also varied as to stage of cutting and moisture content. The per cent of alfalfa was determined for each lot. Buffers were run on the hay put in and pH determinations made of the resulting silage.

A sample of each lot of silage was sealed in an air-tight container and placed in the center of each lot in the silo. Carotene determinations are being conducted on the sealed samples, on a sample taken from the center of each

lot and on a sample of artificial dried hay similar to that which was ensiled in each lot. The results thus far indicate that the sealed samples were high in carotene while the carotene content of the samples from the silo and those artificially dried were much lower. The loss of carotene is no doubt due to the presence of oxygen. In addition the per cent moisture, protein, and total fermentable carbohydrates were determined for the hay put in and the silage fed out.

All lots were fed to eight dairy cows, feeding trials covering four 30-day periods. In the first and third periods the silage was fed as replacement for 20 pounds of hay while in the second and fourth periods the silage replaced 15 pounds of the hay in the ration. The feeding trials show the palatability of the various lots and results of the different feeding periods give some indications of the most practical method of feeding the silage.

When ensiled the lots varied from 72 to 80 per cent legumes. The moisture content varied from approximately 40 to 80 per cent. The per cent protein varied from 13.56 to 16.88. Preservation of carotene varied with the different lots.

This is a preliminary report of an experiment that will be completed May first.

**P12. The Influence of the Quality of Protein in the Concentrate Mixture on the Production of Dairy Cows Fed Mixed Hay and Corn Silage.** G. W. SALISBURY AND F. B. MORRISON, Cornell University.

Numerous experiments have proven that for swine and poultry, or for rats (used as laboratory test animals), the quality or kind of protein in a ration may be fully as important as the amount. Thus far but little information is available as to whether or not the quality of protein is of similar importance in dairy rations composed of the common feeds.

Corn gluten feed and corn gluten meal, which are common dairy feeds, both furnish protein of low quality for swine and rats. Corn gluten feed is often the cheapest protein supplement for northeastern dairymen. It is important, therefore, to determine whether the quality of protein is a limiting factor for high-producing dairy cows fed a ration made up of the common roughages grown in the northeast, the cereal grains, and corn gluten feed or other corn by-products as the only protein supplement.

During the winters of 1935-'36 and 1937-'38 experiments were conducted at the Cornell Station in which this question was studied. Each year two groups of nine cows of nearly equal productive capacity were selected. The cows were all fed mixed hay and corn silage. In 1935-'36 the hay was of better quality and contained more clover than the hay which was fed in 1937-'38.

One-half of the cows each year were fed a "low-quality protein" concentrate mixture composed of ground yellow corn, ground oats, corn gluten feed, corn gluten meal, bonemeal and salt.

The other cows were fed a "high-quality protein" concentrate mixture. It was composed of ground yellow corn, ground oats, soybean oil meal, corn gluten feed, linseed meal, dried distillers' corn grains, cottonseed meal, bone-meal and salt.

*Results.* Some concern was felt when the experiment was planned concerning the palatability of the "low-quality protein" mixture. However, no difficulty was experienced until the 13th week of the first experiment. Then two cows, receiving rather large amounts of concentrates because of their high production of milk went off feed. Up to and including this, the cows on the "low-quality protein" ration had produced 96.2% as much milk as the other cows.

When the "low-quality protein" group were fed the "high-quality protein" concentrate mixture the appetite of the two cows improved greatly. They also increased in production. These results suggested that a nutritive deficiency had become apparent in the "low-quality protein" ration after it had been fed for 13 weeks.

The experiment conducted this winter was similar except that the mixed hay contained a considerably smaller proportion of clover. Up to the present time (the end of the 15th week of the experiment) the cows on the "low-quality protein" ration have produced 107.0% as much milk as the cows on the "high-quality protein" ration. On no occasion have the cows refused the "low-quality protein" concentrate mixture.

**P13. The Influence of Fineness of Grinding on the Coefficients of Digestion on Dairy Cows. T. M. OLSON AND G. C. WALLIS, South Dakota State College.**

The project herein reported was concerned primarily with the effect of fineness of grinding on the coefficients of digestion, and did not consider the effect of grinding grain on the cost of grinding, nor its effect on the production of the cows.

Four lactating cows were chosen and placed in special stalls where the digestion trials were conducted in the regular manner. The six trials were for 14 days with a 7-day preliminary period. The ration consisted of equal parts by weight of corn and alfalfa. The first, third and fifth trials medium fine corn was fed, in the second trial finely ground corn was fed, in the fourth trial whole corn was fed, and in the sixth trial alfalfa alone was fed.

The results with corn indicated that the coefficients of digestion for the entire ration were somewhat higher for the finely ground than for medium ground corn. The coefficients ranged from 1 to nearly 3 per cent higher for each nutrient, including the dry matter.

When the coefficients for the corn alone were computed, the difference in the finely and medium ground corn ranged from approximately 6 per cent in case of ether extract to 27 per cent for the fiber in favor of the finely ground.

The coefficients of digestion of the whole corn in the whole ration was appreciably lower than the ground corn with every nutrient, except crude fiber, ranging from 5 per cent for crude protein to 10 per cent for ether extract.

**P14. The Relation of Certain Succulent Roughages to the Color and Flavor of Milk.** H. H. TUCKER, O. F. GARRETT, C. B. BENDER, New Jersey Agricultural Experiment Station.

The results of the work at this station in 1936 and 1937 indicated that there was a good correlation between high yellow color and good flavor in milk. The results also indicated that molasses grass silage might be an excellent feed for producing milk with high yellow color. Accordingly experiments were designed in 1937-1938 to observe the effect of certain succulent roughages—beet pulp, corn silage, molasses grass silage, carrot-corn silage on color and flavor.

Two separate reversal feeding trials were conducted for the purpose of studying color. In the first experiment corn silage and poor quality field cured hay, grass silage and the same hay, and grass silage as the sole roughage were studied in which the following results were obtained. The color readings for each of the rations based on the mean of the color readings for the last week of each feeding period were, corn silage and hay 5.36, grass silage and hay 5.74, and grass silage 5.85. This means an increase in color of 7.71% in the milk produced on grass silage and hay over corn silage and hay, and 9.14% increase when grass silage was used as the sole roughage as compared with corn silage and hay.

In a second experiment corn silage and hay and grass silage and hay were compared with beet pulp and hay. Each feeding period was for 3 weeks with a one week transition period between changes of feed. A ration of beet pulp and hay was used at the start and end of the experiment. When the last week of each feeding period was compared with the last week of the previous depletion period, there was a color increase from 4.494 to 4.985 or 10.9% for corn silage and hay, and an increase from 4.271 to 5.190 or 21.5% for grass silage and hay.

Carrot-corn silage made from 1 part green carrots and tops and 3 parts green corn was fed to both groups following the completion of the above experiment in March 1938. This silage when fed with field cured hay increased milk color from a beet pulp value of 4.95 to 5.23 or 5.65% at the end of 3 weeks. All cows showed a gain in milk color, with greatest gains made by cows producing milk with lowest color.

The results of the experiment on flavor show that molasses grass silage is definitely superior to both corn silage and beet pulp and that corn silage is no better than beet pulp in this respect. The average flavor score for fresh

raw milk from individual cows for grass silage was 22.28, for corn silage 20.84 and for beet pulp 20.89.

There was also a definite association of high yellow color and good flavor.

**P15. The Effect of the Level of Feeding Dairy Cows Upon the Flavor of their Milk.** J. C. HENING AND A. C. DAHLBERG, New York Agricultural Experiment Station.

As an outgrowth of a cooperative experiment with the United States Department of Agriculture, a study was made of the effect of different levels of feeding upon milk flavors.

The cows were divided into six groups. One group as a control was fed at the Morrison standard and the other groups were fed at levels of 20 and 10 below and 10, 20 and 30 above the Morrison standard. The judging of the flavor of the milk from all of the cows in the experiment station Jersey herd, whether they were included in the cooperative experiment or not, was continued for one year. The raw milk was judged for flavor shortly after milking and the raw, pasteurized, and pasteurized plus copper milks were judged the first, third and fifth days after milking and pasteurization.

The study of the flavors for a complete year offered opportunity to note the effect of seasonal variations on the development of oxidized flavors. Six heifers freshened during the year and the flavor of their milk was compared with that of mature cows.

The results indicate, that, the flavor of the milk or the percentage of occurrence of oxidized flavor was not influenced by the level of feeding under the conditions of the experiment.

The occurrence of oxidized flavor, as has been shown by others, was considerably less in summer months than in winter months but there was not a direct correlation between the period of feeding green cut legumes and the occurrence of oxidized flavors.

The milk from first calf heifers showed a higher evidence of oxidized flavors than milk from older cows but the data was not considered sufficient to warrant definite conclusions being drawn.

**P16. A Study of Some of the Physico-Chemical Effects of Soybeans on the Fat in Cows Milk.** R. W. BRATTON, W. F. EPPLÉ, J. W. WILBUR AND J. H. HILTON, Purdue University.

Previous investigations at this Station have shown that raw soybeans, when fed in amounts equal to 25 per cent of the grain ration, effect a measurable increase in the fat test of the milk produced.

It seems logical, therefore, that this change in fat test would be accompanied by changes in the numbers of fat globules present or their size. Furthermore the chemical composition of the butterfat might change with the variation in the fat test.

A feeding trial has been in progress with the view in mind of determining some of the physical and chemical manifestations of this change in fat test.

Two cows were fed progressively on experimental rations consisting of one part corn, one part oats, and two parts roasted soybeans; one part corn, one part oats, and two parts raw soybeans; one part corn, one part oats, and enough soybean oil meal and soybean oil to make the protein and fat levels approximately equal to those of the raw soybean ration. The control ration consisted of one part corn, one part oats, and 1.5 parts soybean oil meal. Good quality alfalfa hay was fed as the sole source of roughage.

Microscopic examinations of the milk have yielded no conclusive evidence as to the probable effect of soybeans on either the total number or size of the fat globules. Sizes of the fat globules ranged from .1 to 15 microns in diameter, with the mean size between 1.5 and 3 microns. The total frequency ranged from 1.4 to  $4.6 \times 10^9$  per ml.

Chemical analyses of the milk fat, including the iodine number, the thiocyanogen number, and the Reichert Meissl number, have yielded some information regarding the chemical nature of the increase in fat test.

The following table gives the average fat tests, the iodine and thiocyanogen numbers of the milk fat, and the calculated percentages of linoleic and oleic acid produced when the various soybean rations were fed.

Ration	% fat	I-no.	SCN-no.	% linoleic	% oleic
Soybean oil meal (check)	3.36	39.4	36.4	3.3	37.4
Soybean oil meal + soybean oil . . . . .	3.58	45.8	41.7	4.5	42.1
Roasted soybeans . . . . .	3.81	51.0	43.5	8.3	40.3
Raw soybeans . . . . .	*3.88	43.2	39.1	4.5	39.2

\* This slight increase in test over that secured when roasted beans were fed was caused primarily by an abnormally high test of one cow while she was sick during a period when raw soybeans were fed.

The figures in the above table indicate that the increase in fat test secured by feeding either raw or roasted soybeans was of about the same magnitude, but the composition of the resulting fat was noticeably different.

It is interesting to note further that when soybean oil was fed, the increase in test was not so great as when either raw or roasted soybeans were fed, but the composition of the fat resembled that produced by feeding raw soybeans.

The Reichert Meissl numbers which were determined, decreased when the iodine numbers increased. This change accompanied the increases in fat tests and indicates a diluting affect on the glycerides of the volatile fatty acids by some other fatty acids.

Additional studies are being made to determine if the increase in test is due to the oil in the soybeans or some other factor in the soybeans.

**P17. The Vitamin D Content of the Milk Produced by Jersey and Holstein Cattle Receiving the Same Vitamin D Intake.** G. C. WALLIS, So. Dak. State College.

The paired-feeding method is being employed with representative animals from the Jersey and Holstein breeds. Each animal is fed vitamin D at a known and constant level throughout the lactation period from natural food sources, chiefly alfalfa hay. The food intake is controlled so that each animal in a pair received the same amount of vitamin D. Butterfat samples representing the milk for a 4-6 day period are obtained from each animal at monthly intervals throughout the lactation period for vitamin D assay. The project will be continued until data is available from three or four pairs of animals. In this paper the results from one Jersey and one Holstein, which constitute the first pair, are being reported.

The alfalfa hay contained 1588 I.U. of vitamin D per pound. It was fed to each cow at the rate of 12 pounds per day making a vitamin D intake of approximately 19,000 I.U. daily throughout the lactation period. The vitamin D potency of the butterfat from the Holstein cow decreased from 0.43 I.U. per gram in the early part of the lactation to about 0.25 I.U. near the end. The Jersey showed the same tendency but the potency was always higher being 0.63 I.U. per gram in the flush of the lactation and 0.43 at the close. The decrease in the potency of the fat was practically counterbalanced in both cases by the increase in the percentage of fat in the milk as the lactation progressed so that the vitamin content per quart of milk was strikingly uniform throughout the lactation. The vitamin content of Jersey milk fluctuated between 27 and 33 I.U. per quart whereas the Holstein milk varied between 8 and 12 I.U. per quart. However, because of the larger milk production of the Holstein cow, the total amount of vitamin D recovered in the milk was practically the same for both cows. Of the 19,000 I.U. in the feed, the amount recovered decreased from about 1.8 per cent in the early part of the lactation to about 0.50 per cent toward the end.

**P18. Plasma Magnesium Studies on the Growing Bovine.** C. W. DUNCAN AND C. F. HUFFMAN, Michigan State College.

Determinations of magnesium were made on the blood plasma of 107 normal calves at intervals of 1 to 2 weeks over a period of 3 years and values were obtained for the mean concentration of magnesium for the first 18 months of life. The magnesium value showed fairly close agreement from month to month and a definite tendency to increase up to 12-13 months of age. The change in level was accompanied by a series of rhythmic variations which extended over several months. The mean value from the 2286 observed values was  $2.414 \pm 0.005$  mg. per 100 cc. of blood plasma (range 1.62-3.83 mg.) and 79.7 per cent of the values were between 1.895 and 2.795

mg., whereas 72.5 per cent of all of the values actually occurred within the limits established by  $\pm 1$  standard error of prediction.

The same magnesium value recorded above were rearranged, without respect to age, into the respective calendar months in which they were taken and the influence of the astronomical seasons noted. Straight lines were fitted to the 6 means of January-June, inclusive, and July-December, inclusive, and the slope of each line represents the general path of plasma magnesium throughout the year. From November through April there was very little change in the mean but during May and June there was a rapid and steady decline. From July to November the downward trend was reversed and a steady increase in the concentration of magnesium took place. This decline and increase is particularly striking in view of the fact that some of the values were obtained from calves which did not have access to sunlight and were receiving different types of rations, so that the fluctuations in plasma magnesium are not referable to seasonal changes in the ration.

It has been shown that the concentration of magnesium can not be regarded as constant. The range of the so-called normal variation is sufficiently wide to include many variations that occur under pathological and physiological conditions. It is also evident that fluctuations in the plasma magnesium content of the blood of growing calves are to be expected as normal occurrences.

**P19. The Normal Concentration of Inorganic Phosphorus in the Blood of Lactating Dairy Cows and Factors Affecting It. A. H. VAN-LANDINGHAM, H. O. HENDERSON AND G. A. BOWLING, West Virginia Agricultural Experiment Station.**

Inorganic phosphorus has been determined on more than 200 composite samples of whole blood taken from twenty-two Holstein dairy cows at eight-week intervals over a period of two years.

The animals were divided into three groups depending upon the type of ration and management. All rations consisted of a grain mixture, supplemented with 2% bone meal, and alfalfa or timothy hay with or without corn silage. These rations were calculated to supply liberal amounts of digestible crude protein, total digestible nutrients, calcium and phosphorus.

There was no appreciable change in the inorganic phosphorus content of the blood throughout the first lactation, but there was a considerable drop during the last months of gestation preceding the second lactation period. The inorganic phosphorus in the blood was low during the first three months of the second lactation period. Again there was a drop in blood composition during the latter months of pregnancy preceding the third lactation period. During the first three months of the third lactation the blood phosphorus was low, but during the fourth to fifth months it rose to about the normal level followed by a slight decline throughout the remainder of the period.

There was a considerable decline in blood phosphorus from the first to the third lactation after which there was no appreciable change in blood composition. Sixty-two composite samples of whole blood taken during the first lactation averaged 4.95 mgs. per 100 mls., 51 samples taken during the second lactation averaged 4.81 mgs., 31 samples taken during the third lactation averaged 4.35 mgs., and 27 samples taken after the third lactation averaged 4.33 mgs. of inorganic phosphorus per 100 mls.

There was a slight negative correlation between the inorganic phosphorus content of the whole blood and the average daily milk production in pounds for the entire lactation period.

The inorganic phosphorus in the blood of lactating dairy cows was slightly lower during the winter and early spring than during the summer and early fall. This difference was not related to feed or management of the animals.

**P20. The Carotene Content of Market Hays and Corn Silage as Determined by a Quantitative Adsorption Procedure.** LEO A. SHINN, HERBERT G. WISEMAN, EDWARD A. KANE AND C. A. CARY, Bureau of Dairy Industry, United States Department of Agriculture.

When the pigments that occur with the carotene in the feed are separated from it by a modification of the Willstätter and Stoll procedure, using 92 per cent  $\text{CH}_3\text{OH}$  in the removal of the xanthophyll, the carotene content in milligrams per kilograms with U. S. No. 1 alfalfa hay varies from 19 to 121, average 43; with U. S. No. 3 alfalfa, from 1 to 11, average 4.5; with U. S. No. 1 timothy hay, from 8 to 36, average 21; with U. S. No. 3 timothy hay, from 1 to 12, average 5.5; and with corn silage, from 1 to 40, average 13.7.

With none of these materials does the spectral absorption curve for the pigments in the "carotene" fraction correspond with that of  $\beta$ -carotene within the limit of experimental error. The absorption is relatively too high at wave lengths shorter than 450  $\text{m}\mu$ , and too low at wave lengths longer than this to correspond with this carotene.

If these carotene fractions are filtered on adsorption columns in such a way as not to destroy any of the pigments in them, two colored fractions may readily be obtained—one with the spectral absorption of  $\beta$ -carotene and the other, probably made up of several pigments, with a spectral absorption that would account for the discrepancy between that of the original unfiltered sample and  $\beta$ -carotene; and the amount of carotene obtained in this way from the original, unfiltered carotene fractions is the same as that calculated, from the spectral absorption curve of  $\beta$ -carotene and that of the impurity removed by filtration, to be present in them. An adsorption procedure has, therefore, been devised for the routine determination of carotene in feeds. For U. S. No. 1 alfalfa hay, it gives results on an average of 18

per cent lower than those above; with No. 3 alfalfa, about 39 per cent lower; with U. S. No. 1 timothy hay, 25 per cent lower; with No. 3 timothy hay, about 40 per cent; and with corn silage, 30 per cent lower. The above figures, corrected accordingly, probably represent more nearly the vitamin A potency of these feeds.

**P21. Relationship Between Carotene, Blindness Due to Constriction of the Optic Nerve, Papillary Edema and Night Blindness in Calves.** L. A. MOORE, Michigan Ag. Exp. Station.

For the past 20 years various experiment stations have noted blindness in calves associated with rations where poor quality or no roughage was fed. The condition is also associated with poor reproduction. Previous work from Michigan has shown that the blindness is associated with a constriction of the optic nerve where it passes through the optic foramen. Further work with calves has shown the blindness is preceded by papillary edema and generally by night blindness.

Excellent work from California has shown the association of vitamin A and night blindness in cattle. Various workers have associated vitamin A deficiency and blindness due to constriction of the optic nerve although no direct proof has been produced. Two Holstein calves placed on a vitamin A deficient ration (a ration which had previously produced the blindness) and fed crystalline carotene dissolved in cottonseed oil in quantities sufficient to keep the blood plasma carotene level well above a lower limit of 0.13 gammas per cc. have not developed night blindness, papillary edema or blindness due to constriction of the optic nerve. It therefore appears that vitamin A deficiency is the cause of the development of these conditions.

In mature cows night blindness and papillary edema develop but blindness due to constriction of the optic nerve does not develop due to the fact that the optic canal is well calcified and is not growing in length.

In calves blindness due to constriction of the optic nerve and papillary edema are associated while night blindness is a separate process but due to the same deficiency.

**P22. The Carotene Requirements for Normal Reproduction.** H. T. CONVERSE AND EDWARD B. MEIGS, Bureau of Dairy Industry, United States Department of Agriculture.

In 1936 Meigs and Converse reported before this society the results of their work on the carotene requirements of dairy cattle for reproduction and lactation. The roughages which furnished the carotene for the animals used in that work constituted approximately half the ration. With U. S. No. 1 alfalfa hay there was a normal number of normal calvings; with U. S. No. 3 alfalfa or timothy hay, all calvings were abnormal where the animals had been on these rations for 5 months or more; and it was reported that with

U. S. No. 1 timothy hay or U. S. No. 1 clover hay (or clover light timothy mixed) less than 50 per cent of the calves were normal at birth. The average daily carotene intake of the cows on the No. 1 timothy and No. 1 clover rations was probably about 50 milligrams per animal or 100 micrograms per kilogram of body weight. This was calculated by using average figures for these two grades of hay. As there is a wide variation in the carotene content of hays of the same grade, and as considerable loss of carotene occurs during their storage, it seemed desirable to obtain more definite information on the effect of hays supplying approximately this apparently critical level of carotene.

We have, therefore, noted the carotene intake, during the last 3 months of gestation, of all cows on rations of grain and No. 1 timothy hay or grain and No. 1 clover or clover mixed hays. Seven calvings have occurred. All have been apparently normal. The carotene intake was from 80 to 130 milligrams daily or from 178 to 260 micrograms per kilogram or body weight. Two other cows on a ration containing clover hay had a daily carotene intake for the last 3 months of gestation of about 30 milligrams (60 to 70 micrograms per kilograms of body weight) gave birth to very weak calves, though one did survive. One first-calf heifer on timothy hay received about 90 milligrams of carotene daily at the start of gestation and about 42 milligrams daily at calving. This carotene intake represented 340 micrograms per kilogram of body weight at conception and 114 micrograms per kilogram at calving. The calf was born dead. As previously reported, no cows on our lower grades of timothy hay have calved normally.

Our amounts of carotene for safe calving are therefore somewhat larger than those reported by Guilbert and Hart of California.

We conclude that for normal calving, cows should receive during the last months of gestation 80 to 100 milligrams of carotene daily. If the daily carotene intake is as little as 60 milligrams per cow there will probably be a considerable proportion of dead calves.

**P23. Vitamin A for Growth and Reproduction in Dairy Heifers. I. R. JONES AND J. R. HAAG, Oregon State College.**

The possibility of dairy heifers being fed rations too low in vitamin A for good growth, normal reproduction and well-being has been indicated by the investigations of several workers in recent years.

In the fall of 1936, thirty purebred dairy heifers were placed on a basal ration consisting of poor quality oats and vetch hay and a grain mixture composed of barley, oats, wheat bran, linseed oil meal, bone meal and salt. The oats and vetch hay was quite mature when cut and was rained upon and bleached during the curing process. This hay was found to contain about nine parts of carotene per million. Preliminary vitamin A assays with rats gave results of essentially the same order.

The heifers, ranging in age from 6 to 20 months and previously maintained on good rations, were divided into two similar groups, one of which was fed in addition to the basal ration, a vitamin A supplement in the form of salmon oil at the rate of 5 c.c. per hundred pounds live weight daily. Rat assays indicate that the salmon oil has a vitamin A potency of about two times that of U. S. P. XI Reference Cod Liver Oil.

All heifers were fed individually and accurate records kept of feed consumption, breeding behavior, health and growth. The heifers of both groups continued to grow at essentially the normal rate during the 6 months' period.

The reproduction records of the two groups of heifers have been fairly normal with a possible benefit to be attributed to the salmon oil feeding. Three heifers receiving salmon oil and 2 controls successfully continued pregnancy from about the fourth month until 3 to 4 weeks before calving when they were given a better quality hay and corn silage. Five salmon oil fed heifers and 2 controls became pregnant while receiving the experimental rations. Delay in estrus and failure to conceive at service were somewhat more prevalent in the control group.

The heifers that had not freshened were pastured for 6 months in the summer of 1937. Five heifers calved normally during this period. Five salmon oil and 11 control heifers became pregnant while on pasture.

In the fall of 1937, fourteen of the original heifers, 7 in each group, were again placed on similar experimental rations. Also, an additional 24 heifers averaging 9 months of age, were paired into two groups. The hay fed in 1937-38 was a mixture of poor quality oats and vetch and sudan grass with an average carotene content of about 11 parts per million.

After 4½ months' feeding, the weights and heights of all animals are normal. The heifers bred while on pasture have successfully completed pregnancy. Other reproduction records are incomplete. No harmful effects have been observed as the result of feeding as much as 60 c.c. of salmon oil daily.

The experimental results would seem to indicate that a comparatively low vitamin A ration will not result in serious disturbances in dairy heifers when fed over a period of about six months either preceded or followed by a pasture period.

**P24. The Value of Dried Molasses and Yeast for Dairy Calves. O. L. LEPARD, P. E. NEWMAN AND E. S. SAVAGE, Cornell University.**

In view of the introduction of dried molasses and yeast as a dairy feed and previous published results concerning the value of cereal yeast for dairy calves, studies were made to determine the relative replacement value of dried molasses and yeast for skimmilk in dairy calf starters.

Two lots of 5 calves each were raised by the calf starter method. Each calf received 350 lbs. of whole milk allotted over the first 7 week period. Calf starter was fed free choice to a maximum of 4 lbs. per day for the first

16 weeks. As soon as the calves reached 4 lbs. of calf starter a heifer ration was fed to a maximum of one pound per day until the calves were 16 weeks of age. At 16 weeks of age the calf starter was discontinued and the heifer ration fed to a maximum of 5 lbs. per day for the remainder of the trial. Alfalfa heavy grass mixed hay, U. S. Grade No. 2, was fed free choice throughout the experiment. All experimental trials were of 20 weeks' duration.

To test the value of dried molasses and yeast as an ingredient in calf starter one group was fed 5% dried molasses and yeast replacing an equal amount of dried skim milk in a previously tested formula containing 20% dried skim milk. The other group was fed 7.5% dried molasses and yeast replacing 5% of dried skim milk in a calf starter formula containing 10% dried skim milk.

The group receiving 5% dried molasses and yeast consumed 227.1 lbs. of total digestible nutrients per 100 lbs. of gain as compared with 238.5 lbs. of total digestible nutrients for the check group containing no yeast. The average daily gains from 2 weeks of age for this group was 121.6% normal as compared to 110.1% normal for the check group.

The group receiving 7.5% dried molasses and yeast consumed 231.9 lbs. of total digestible nutrients per 100 lbs. of gain as compared with 254.1 lbs. of total digestible nutrients for the check group containing no yeast. The average daily gains from 2 weeks of age for this group was 118.7% normal as compared to 112.2% normal for the check group.

All calves appeared normal and in a thrifty growing condition throughout and at the end of the experimental periods.

Dried molasses and yeast is hygroscopic in nature and contains 32% mineral ash. Each of these factors may offer some difficulty when large amounts are used in a ration.

**P25. Initiation of Lactation in the Albino Rat.** R. P. REECE, New Jersey Agricultural Experiment Station.

A number of investigators have reported the failure to initiate lactation in the albino rat, either with an anterior lobe extract or with lactogen. Two possibilities have existed to account for this non-functional responsiveness: first, that the experimentally developed mammary glands of the albino rat were not developed to a state entirely comparable to that which takes place during pregnancy and secondly, that the albino rat was non-responsive to lactogen.

This problem has been re-investigated. Pseudo-pregnancy was induced in rats by injecting a gonadotropic hormone. On the 12th day following the induction of pseudo-pregnancy the animals were ovariectomized and a mammary gland removed. Experimental animals were then injected with pituitary glands, anterior lobe acetone dried powder or lactogen every eight

hours until six injections had been made. Three or four hours following the last injection the animals were sacrificed and their mammary glands observed macroscopically and microscopically. Control animals were employed also and they received the same treatment as experimental animals except that no injections were made following ovariectomy.

Lactation was observed in none of the control animals. In the experimental animals a small amount of milk was observed following the injection of lactogen, pituitary glands from spayed female rats and pituitary glands from normal rats. A fair amount of lactation was observed in experimental animals following the injection of pituitary glands from normal and ovariectomized rats which had been injected previously with estrogen. In not one case was lactation observed which was comparable to which occurs in the parturient female rat.

A group of pseudo-pregnant rats were then injected daily with 200 i.u. of estrogen during the pseudo-pregnancy period. The experimental animals were then injected with an acetone dried powder made from cattle pituitaries. In all cases lactation was observed which was comparable to that observed in the rat following parturition. Lactogen injections into similarly treated rats resulted in inconsistent results.

These observations demonstrate that the mammary glands of rats are functionally responsive to injected pituitary tissue and emphasize again the importance of anterior pituitary hormones, other than lactogen, in initiating lactation.

**P26. Recent Advances in Our Knowledge on the Endocrine Control of Mammary Development.** E. T. GOMEZ AND C. W. TURNER, Missouri Agricultural Experiment Station.\*

Until recently, development of the mammary gland had been assumed to be stimulated directly by ovarian hormones. Gomez, Turner and Reece (1937) first demonstrated, that while normal development of the mammary gland could occur only in the presence of functional ovaries, the action of ovarian hormones (estrogen and progestin) is indirect, by way of the anterior pituitary gland. These observations have since been confirmed and extended to several species of laboratory animals (Gomez and Turner, 1937-1938). Studies with hypophysectomized rats, rabbits, cats, and guinea pigs maintained under strict hygienic regimen and nutritive care have shown that administration of estrogen and/or progestin daily for periods ranging from 20 to 30 days was ineffective in stimulating growth of the mammary gland. Similarly, administration of somewhat purified preparations of anterior pituitary hormones, including the thyrotropic, adrenotropic, gonadotropic,

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and lactogenic hormones; the thyroid (thyroxin) and adrenal cortical (eschatin) hormone; and acetone dried or fresh anterior pituitaries of sheep alone and in combination with estrogen or estrogen and progestin was ineffective in stimulating the growth of the mammary gland. These observations were interpreted as indicating that estrogen and/or progestin stimulates the production of a specific mammary gland growth-promoting hormone which in turn exerts a direct action upon the mammary gland. This hormone entity has been called "Mammogen." According to the above hypothesis, the pituitaries of animals during estrum and pregnancy or those which have been receiving estrogen treatments should contain a high concentration of this mamnogenic hormone. Sufficient evidence has now been accumulated to confirm and extend this hypothesis.

Implanation of one adult male rat pituitary daily for 20 days produces extensive arborization of ducts and proliferation of the lobule-alveolar system of the mammary gland of hypophysectomized immature male and female guinea pigs. The donor rats have previously estrogen treatments daily for 10 to 20 days. On the other hand, implanation of one adult normal male rat pituitary daily for 30 days is effective in stimulating the mammary glands of hypophysectionimized guinea pigs. Daily administration of 50 mgs. of macerated anterior pituitary from pregnant cattle for 20 days or more causes the growth of ducts and proliferation of the lobule-alveolar system of the mammary gland of gonadectomized immature female rats and rabbits. This growth is comparable to that observed in late pregnancy.

*Summary.*—The normal development of the mammary gland is under the direct influence of a "mamnogenic hormone entity" of the anterior pituitary gland. This hormone entity is elaborated in ample amount by the pituitary gland, to produce physiological effects upon the mammary gland following injection of ovarian hormones or during pregnancy.

**P27. The Biological Assay of "Mammogen."** A. A. LEWIS AND C. W. TURNER, Missouri Agricultural Experiment Station.\*

The normal male mouse at about puberty (10 to 25 grams body weight) has 8 to 10 very rudimentary mammary glands, without teats. These consist of one to three thin ducts with perhaps a few short side-branches.

Subcutaneous injection of fresh, macerated anterior lobe tissue from pregnant animals in water in the lumbar region of normal male mice (castration is unnecessary) once daily for a minimum of four days has been found to stimulate the mammary glands to the development of thick ducts, numerous side branches, and large club-like, dark staining end-buds. Not all of the glands on a mouse skin will develop to the same extent. Those at the anterior end usually respond most readily.

\* Contribution from the Department of Dairy Husbandry, Missouri Agr. Exp. Sta., Journal Series No. 554. Aided in part by a grant from the International Cancer Research Foundation.

The total dosage necessary to secure such development can be reduced by injecting smaller amounts daily for six days. The mice are then killed on the seventh day and the skins mounted on cork and placed in a fixative. The interior fascia is later removed, stained, and examined under a binocular microscope. One or more well developed glands with large end-buds, denoting rapid growth, is considered a positive response. Because of variability in the mice, response should be secured in  $50 \pm 10$  per cent of ten mice.

Formulative of an assay technique is facilitating determination of comparative concentrations of "mammogen" in different extracts, in the pituitaries of different species, and at different stages of pregnancy. Work is also progressing on methods of chemical extraction and purification. The response to "mammogen" of other mammalian species is also being investigated.

*Summary.*—The male mouse, of between 10 and 25 grams body weight, which invariably has very immature glands, has been found to be an excellent assay subject for the mammogenic factor of pregnancy pituitaries. The proposed assay unit is the total amount of pituitary tissue or extract, injected subcutaneously for six successive days, necessary to secure pronounced end-bud development on at least one gland each of  $50 \pm 10$  per cent of ten normal 10 to 25 gram male mice.

**P28. Milk and Fat Production of Dairy Cows as Influenced by Thyroxine, and Anterior Pituitary Extracts.** NOEL P. RALSTON AND H. A. HERMAN, Missouri Agricultural Experiment Station.\*

Recent experiments at this and other stations have demonstrated an augmentation of milk and fat secretion when cows are administered thyroxine, and extracts of the whole anterior pituitary body. In general, previous experiments have been of short duration, but have served to further emphasize the relation of the endocrine secretions with respect to the functioning of the mammary gland.

The present project has as its objective (a) a further study of the influence of thyroxine and other thyroid substances on milk and fat secretions; (b) the effect of extracts of known potency from the whole anterior pituitary, on milk and fat secretion; and (c) the relative effects of some of the fractions of the anterior pituitary on milk secretion. Included in this study is the determination of the minimum dosage of the respective hormone substances required to bring about a maximum response in increased milk and fat yield at the various stages of lactation. Cows in the various stages of lactation, and of high, low and intermediate producing ability have been selected for these experiments.

Monthly injections of thyroxine, given in three successive doses of 10 to 15 mg. daily, have been found to give an increase of 10 to 20 percent in milk

\* Contribution from the Department of Dairy Husbandry, Missouri Agr. Exp. Sta., Journal Series No. 562.

yield. The maximum increase is usually attained within five to six days following the beginning of thyroxine administration. Milk secretion continues at an elevated level for 10 to 12 days following the treatment and gradually returns to the pre-injection level. The fat percentage has increased rather markedly with a slight increase in solids-not-fats. Cows in the early decline of lactation appear to give the most pronounced increase in yield.

Preliminary work with whole anterior pituitary extracts demonstrates that large doses are necessary to obtain an increase in milk yield. However, the size of the dose varies considerably with different cows and with the stage of lactation. Cows in the early stages of lactation have shown indications of making the greatest increase in yield following treatment.

The work at present has not progressed to the extent of permitting definite conclusions as to the maximum response obtained by uniform dosages of these hormone substances administered at the various stages of lactation.

**P29. Vitamin C Metabolism in the Dairy Cow.** W. H. RIDDELL AND C. H. WHITNAH, Kansas Agricultural Experiment Station.

It has previously been reported from this station that the change from winter feeding to spring pasture did not increase the vitamin C content of milk. What effect then has the increased intake of vitamin C on the vitamin C metabolism of the dairy cow?

The following effects were noted in three cows which were changed from a winter ration to all the green rye they would consume.

1. The vitamin C intake increased regularly for ten days to a maximum of over sixty grams per day.
2. The average vitamin C content of the blood more than doubled within twelve hours after the green feed was first supplied. The blood level reverted to normal within the next twelve hours.
3. The average output of vitamin C in the urine was increased over five-fold within sixty hours after green feed was first supplied.

The fate of vitamin C was studied in the rumen contents of a cow with a rumen fistula, and in a steer at slaughter. In each case the rumen contents contained less than one-tenth the vitamin C of grass ingested twelve hours earlier. The solid and liquid portions of any sample of the rumen contents contained equal concentration of vitamin C.

The early rise of vitamin C in blood and urine, and its rapid disappearance from the rumen, suggest that the vitamin was rapidly absorbed. The first two of these changes also suggest that an ample supply was available even to cows on winter feed. The return of blood to normal levels, the failure of urinary output to continue increasing, and the failure of vitamin C output in milk to increase, all indicate that the increased intake may have been compensated for either by decreased synthesis or by increased destruction.

**P30. Fat Metabolism of the Mammary Gland.** J. C. SHAW AND W. E. PETERSEN, University of Minnesota.

Blood fat arterio-venous differences were determined in numerous experiments. There was a consistent and comparatively large loss in blood fat as determined by Allen's (1934) method. The arterio-venous fat loss did not show any relationship to either arterial blood fat level or quantity of milk secreted. However, the arterio-venous fat differences increased with the increase in period of time following milking.

The arterio-venous fat loss was sufficient to account for all of the fat in the milk as shown by the relation of fat arterio-venous differences to the arterio-venous loss of calcium, and the combined loss of glucose and lactic acid, when it is postulated that some of the fat is used as a source of energy for the gland by oxidation, which in turn will account for the lower fatty acids found in milk fat.

**P31. Enzymatic Relationship to the Synthesis of Milk Fat.** PHILIP L. KELLY, University of Arkansas.

A previous study indicated the presence of sufficiently large amounts of free fatty acids in the bovine mammary gland to suggest that they played a significant part in the actual processes of milk fat secretion. It was thought that their presence might come about by the action of enzymes on the blood fat constituents, and a study was made to determine this point.

Three glands were used in the study. Samples of tissue from each gland were heated in a water bath to over 75 degrees C. for the purpose of stopping enzyme action. These samples served as controls. These and additional samples which were not heated were mixed with portions of ethyl-butyrate and toluene, and allowed to stand at room temperature for a period of not less than five days. They were dried with calcium sulphate and extracted with ethyl ether. The concentrated extract was made up to volume with petroleum ether, and the acid value was calculated from aliquots of this material in each determination. Each analysis was made in duplicate. The necessary blank analysis was determined in each case.

The following table gives the acid values of the mammary gland fat under the various conditions of the experiment.

Gland number	Condition of the gland	Acid value fat of fresh tissue	Acid value fat of heated tissue	Acid value fat of unheated tissue
1. . . . .	Lactating	84.0	38.5	88.2
2. . . . .	Lactating	39.7	54.5	90.5
3. . . . .	Non-lactating	17.4	26.4	154.1

In each instance there were very marked differences in acid value between the samples of heated and unheated tissue. The evidence obtained indicates

that enzyme action may be responsible for the presence of free fatty acid in the secretory tissue of the bovine mammary gland.

**P32. The Effect of Fasting and Refeeding on Milk Secretion in the Cow and Goat.** L. E. WASHBURN, Department of Dairy Husbandry, University of Missouri, Columbia, Missouri.\*

Data on yield of milk and percentage of milk constituents indicate a remarkable persistency to maintain lactation during fasting in the cow and goat. Certain data also show that fasting may have a beneficial effect on later milk secretion. While levels of fasting energy metabolism are approached at the same rate in these animals, declines in milk secretion proceed at different rates. At 72 hours after feed, in the cow, milk yield has declined 50% and fat percentage has increased 100%; in the goat, milk yield has declined 80% and fat percentage has increased about 400%. Milk yield and percentages of constituents return rapidly to original pre-fast values upon refeeding.

**P33. The Course of Fasting Energy Production Curves in the Lactating and Dry Dairy Cow Under Similar Environmental Conditions.** L. E. WASHBURN, University of Missouri.†

Preliminary studies indicate that, under similar conditions of treatment and environment, the fasting energy production of a lactating dairy cow is of the order of 10% higher than that of the dry cow. This difference is quite constant until about 60 hours after feeding. The heat production of both cows declines 47%, and reaches a level at 36–48 hours after feed. While this level is maintained by the dry animal, the heat production of the lactating animal further declines about 16% after 60 hours. Milk yield decreases about 50% during 72 hours of inanition, but the fat production remains at a remarkably constant level.

These studies seem to show that in the dairy cow (1) lactation as a function is maintained within certain limits of inanition; and, (2) the higher energy metabolism of the lactating animal is due in a large measure to specific dynamic effect of the food. It is believed that lactation stimulus, endocrine or otherwise, acts to a considerable degree upon the alimentary system of the animal.

**P34. Nature of Swelling in the Cow's Udder at Calving Time.** W. W. SWEET, C. A. MATTHEWS AND R. R. GRAVES, Bureau of Dairy Industry, U. S. Department of Agriculture.

Opinions differ as to the extent to which the intensity of swelling in the udder at parturition interferes with the normal functions of the mammary

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† Paper No. 165 in the Herman Frasch Foundation Series. Contribution from the Department of Dairy Husbandry, Missouri Agr. Exp. Sta., Journal Series No. 558.

glands and with maximum milk secretion. Recently a cow was slaughtered soon after she had calved, when her udder was in an extremely swollen condition with the cool, plastic kind of swelling in which pressure with the finger tip leaves a persistent indentation. The cow was a slow, hard milker. She produced as much as 74 pounds of milk in one day and a total of 520 pounds of butterfat for the year as a 4-year old. A pronounced udder swelling accompanied by marked abdominal swelling occurred at each of her four calvings. At the fourth parturition the size of the udder reached extreme proportions.

Although the udder was milked out immediately before the cow was killed, the amputated udder weighed 165 pounds. The fluid-holding capacity of the udder was 111 pounds. An incision between the right and left halves showed a layer of swelling about 2 inches thick between the skin and the glandular tissue. The surfaces exposed by the incision indicated that the material making up the swelling consisted chiefly of a clear fluid in which there was a network of fine silky fibers, glistening in appearance, and resembling spider's web. The fluid was tenaciously held and did not escape. The swelling obviously was edematous in character. Histological studies indicated that the swollen material contained an interwoven mass of fibers, and that the swelling did not invade the adjacent glandular tissue to any appreciable extent. All four quarters of the udder were functioning at the time of slaughter and aside from the edematous swelling there was nothing to indicate any significant abnormality.

### P35. Some Factors Affecting the Resistance of Animals to Mastitis.

LLOYD A. BURKEY, EDWARD B. MEIGS, GEORGE P. SANDERS AND MORRISON ROGOSA, Bureau of Dairy Industry, U. S. D. A.

A study was made of the number of leucocytes present in the milk, the germicidal property of the milk and the inhibitory action of bovine blood serum against *Streptococcus mastiditis* in an effort to evaluate these agents as natural resistance factors against mastitis.

Results obtained show that large numbers of leucocytes in milk indicate an injury to the udder, but not necessarily the presence of an infection. Leucocytes appear to act as effective agents against infection in some cows, while in others infection is delayed for a number of months or until a general breakdown in animal resistance takes place.

The germicidal property of the milk differs in individual cows and in the different quarters of the same cow. There is also a tendency for this property of milk to increase with the increase in the severity of the disease and with the increased numbers of leucocytes until the acute stage of mastitis is reached. After this point the milk contains less germicidal substance.

Bovine blood serum contains a factor inhibitory to the growth of *Streptococcus mastiditis* in milk of the same cow in vitro. This factor is ineffective

after the serum is 4 or 5 days old and when added to milk from a cow other than the one providing the serum. The inhibitory power of blood serum decreases rapidly with increased symptoms of mastitis and appears to be intimately related to animal resistance. Blood serum lacking in this inhibitory factor acts as a medium and therefore enhances the growth of *S. mastiditis* in vitro. The combined action of the germicidal property of the milk and the inhibitory factor of the blood serum produces a greater retardation of growth of *S. mastiditis* in vitro than is produced by each separately.

The extent to which these inhibitory factors in vitro are identical in their action in the udder and just how significant they may be as factors in the resistance of the animal to the invasion and growth of *S. mastiditis* permits considerable speculation. It is possible that an injury resulting in the increase of chlorides in the milk may at the same time bring about an inflow of blood serum in the udder. It is conceivable under such a situation, which may be quite common, that this effect would result in either a protection against infection or more favorable conditions for growth of *S. mastiditis* in the udder. Results obtained, when milking machines were used at high vacuum, showed an increase in the percentage of chlorides in the milk and an increase in the incidence of mastitis.

Results obtained in the study of these factors indicate that they may be significant defense agencies against infection. Further studies are needed to determine the manner in which these animal defenses may be fortified by animal management to make them more effective safeguards against infection.

**P36. Preventing Sudan Grass Poisoning.** FREDERICK BOYD, O. S. AAMODT, G. BOHSTEDT AND E. TRUOG, University of Wisconsin.

During the past year a method for determining the amount of prussic acid present in plant tissues has been developed which is simple, fairly rapid, and is believed to be considerably more reliable than some of the other methods which have been proposed. This method may be referred to as a "chloroform-steam distillation-picric acid colorimetric" procedure. By this test several hundred farmers' samples of sudan grass have been analysed for prussic acid during the past year, together with great many samples from experimental plots.

The findings are as follows: Short, dark green sudan grass is at times so high in prussic acid, or hydrocyanic acid, as to be dangerous to pasture. Second growth after pasturing or removal of a hay crop when short and dark green is especially dangerous. Sudan grass which is 1½ feet or more in height is usually low in hydrocyanic acid and is relatively safe to pasture. Sudan grass, short or tall, which is of a pale or yellowish green color is low in cyanide poison and is relatively safe to pasture.

A high level of available nitrogen and a low level of available phosphorus in the soil tend to increase the poison content in plants, while a low level of available nitrogen and a high level of available phosphorus have the opposite effect. A high cyanide content may, however, still occur in short plants, especially in the second growth, even though the level of available phosphorus is high.

Drought probably operates as a factor, largely, by keeping the plants small in which stage they are always much higher in cyanide than when larger.

When sudan grass is dried and made into hay, the cyanide poison content does not change greatly. Since it is usually not cut for hay until it reaches a height of three feet or more, there should be little if any danger from cyanide poison in sudan grass hay.

Cattle when turned into sudan grass of high poison content usually stop eating after about 15 minutes, due to the action of the poison. If the animals are not too hungry and are in a high state of vigor, they usually stop eating before they take a fatal dose. If they are in a low state of vigor and very hungry, they are more apt to eat a fatal dose.

For this reason it is important that cattle be given some other feed previous to turning them into a sudan grass pasture, when this is done for the first time during a pasture season. As an added precaution, it is a good plan to watch the herd for an hour on being turned into a field of sudan grass. If some of the animals stop eating sudan grass after a few minutes, or look around for other grass to eat, it is a good sign that the sudan grass is dangerous.

**P37. A Report of the Occurrence of Four Cases of Agnathia. FORDYCE ELY, H. B. MORRISON AND F. E. HULL, Kentucky Agricultural Experiment Station.**

Agnathia or an agnathic condition, according to Webster, describes a case where the jaw is absent or deformed. During the past two years three male calves have appeared in the Kentucky Agricultural Experiment Station herd, and one in a related herd, whose lower jaw and tongue anterior to first pre-molar are entirely lacking. The calves in the Experiment Station herd were all by the same sire and also closely related through their dams. A genetic analysis is presented showing relationship coefficient between afflicted calves according to formula of Lush.

One of the calves was carried for a normal gestation period, another was discovered in the uterus of a cow slaughtered in another experiment; a third calf was discovered as an aborted fetus from a Bang's disease negative heifer. A fourth agnathic male calf has come to the attention of the authors in a Western Kentucky breeder's herd. The sire was from the Experiment Station herd and the afflicted calf carries a concentration of the same blood as the first mentioned calves.

Other anatomical data on three of the calves are presented. The heads and hearts show identical abnormalities which are described in some detail.

**P38. Maximum Initial Yield and Persistency as Inherited Characters Influencing Total Lactation Yield.** LESTER O. GILMORE, WM. E. PETERSEN AND J. B. FITCH, University of Minnesota.

A review of the literature reveals that there are almost as many opinions against the idea that inheritance affects persistency and initial maximum yield as there are opinions and data in support of it. The data represented in this report were taken from a study of the generally considered influences on persistency. They are presented here because they represent a different type of analysis and because they seem conclusive.

The data were taken from the records of the University herd and from a cow testing association. The former herd is managed under a standard plan, adopted in 1923, in which it is aimed to provide as standard an environment as possible in regard to age of calving, lactation during which cow is on official test, duration of test period, frequency of milking and ration utilized. The C. T. A. records all came from a herd larger than the average in which conditions of good management were known to prevail and in which the usual exigencies of poor crop yields were minimized.

In the analyses there was a breakdown of the data, first according to breeds and secondly according to the sires within the breed. Thirdly, for sires having a relatively large number of daughters, the production of the daughters was grouped according to the sire of the dams of these daughters.

With three-time-a-day milking one group of daughters averaged 19 per cent more milk in a year than did the daughters of another bull. Both groups started out at approximately the same initial yield, the difference consequently being ascribed to the difference in persistency that resulted. One group of a sire's daughters averaged 32 per cent more milk a year than did another group of the same sire's daughters, the grouping being made according to the sires of the daughter's dams. The persistency was similar in the two groups but there was a marked difference in the initial maximum yield. The significance of this difference is increased by the fact that one group of these dams was purchased and the differences between this group of daughters and each of the other groups are consistent. It is known that selection in the herd from which these purchases originated was not based primarily upon production. The smaller differences in initial yield of daughters from cows developed in the University herd show some of the effects of selection of sires over a long period, on the basis of their daughters' production. The graphs presented indicate that hereditary factors affecting initial maximum yield and persistency are inherited independently.

**P39. Herd Averages Computed by the Cow-Year Method Versus Herd Averages Based Only on Cows on Test at Least 10 Months.** JAY L. LUSH AND FLOYD JOHNSTON, Iowa State College.

A survey of Iowa C. T. A. Herds including 114 herd years indicates:

1. Cows entering the herd during the year average more on the cow-year basis than do cows which are in the herd all year. (Presumably their age handicap is more than offset by the absence of dry period and by the fact that it tends to be the flush part of their lactation which is included. Probably the records are higher than the real abilities of the cows.)

2. Cows leaving the herd during the year average less on the cow-year basis than do cows which are in the herd all year. (Presumably some of these have been sick and many have been in the later stages of their lactations. The records are probably poorer than the cow's real abilities.)

3. Cows on test between 10 and 12 months of course average more on the cow-year basis than they do if each such cow is counted as a unit in computing the average.

4. The bias from 1 almost exactly cancels that from 2 when large numbers are involved but of course will not always do that in individual herds.

5. Only about 60 to 70 percent of the total number of cows in the herd at some time or other during the year are in it for more than 10 months of that year. A herd average based only on those cows which were on test at least 10 months will sometimes be erratic because of the small number of animals included.

6. Which method will most accurately indicate the average genetic ability of the herd depends mainly on whether in individual herds the errors from 5 are usually larger than the errors from 1 failing to balance 2.

**P40. Age and its Influence on Culling and Life Expectancy in Dairy Cows.** D. M. SEATH, Kansas State College, and J. L. LUSH, Iowa State College.

In a study of 147 Iowa cow testing association herds for the years 1931 to 1935 inclusive, it was found that an average of 29 per cent of the cows left these herds each year. This included cows leaving for all reasons, *i.e.*, disease, low production, injury, death, dairy purposes, *et cetera*. In classifying the cows for a particular year, those remaining in the herd for the entire year following were called non-culls, while those leaving before the completion of the following year were classified as culls.

Of the cows under eight years of age, the two and three year olds were culled most severely with 28 and 31 per cent respectively of these leaving the herds each year. Only 23, 25, 27, and 26 per cent left the four, five, six, and seven year old groups. Those eight or more years of age showed an increase in percentage culled ranging from 32 to 100 per cent.

The spread between the production of the non-culls and culls was compared for the various age groups in order to obtain evidence of when the most culling took place. When considered on this basis culling for production among younger cows was the most pronounced for the three year olds who

showed a spread between culls and non-culls of 75 pounds of butterfat. The two year olds had a difference of 63 pounds, while those from four to twelve years of age had a narrower spread, ranging from 32 to 60 pounds of butterfat. The milk production differences between the groups showed a similar trend.

Life expectancy estimates in terms of time cows will stay in C.T.A. herds have been computed for cows from two to seventeen years of age inclusive. In their computation, the number of animals falling into the various age groups at the beginning of each cow testing association year was used. The number present for a given age compared to the total cows represented in older groups was used as a basis for estimating their life expectancy. The data for the five years were combined to smooth out irregularities noted for individual years. In the resulting table the three year olds had the longest life expectancy in herds. The four, five, and two year old groups followed in order named. A rather slow decline was noted in the life expectancy up to nine years of age, with a rapid decline thereafter.

**P41. The Breeding Efficiency of Proved (Aged) Sires.** J. R. DAWSON,  
Bureau of Dairy Industry, U. S. Department of Agriculture.

A study was made of the breeding efficiency of 20 proved sires used in eight branch experiment station herds maintained by the Bureau of Dairy Industry. The sires included 8 Jerseys, 2 Guernseys, and 10 Holsteins, and only services and conceptions after the sires were 5 years of age were included.

Fertility is expressed by the percentage of the services to fertile cows that resulted in conceptions. The effect of extra services and services to unfertile cows is considered separately. A total of 3,585 services were included in the study, of which 2,982 were to fertile cows. The data were tabulated and analyzed from the standpoint of (1) the relative fertility of the individual sires, (2) the effect of advancing age on fertility, (3) the effect of frequency of service on fertility, (4) the effect of season of the year and climatic conditions on fertility, and (5) the effect of moving sires on fertility.

Seventeen of the 20 sires averaged 4 years and 5 months of fertile service in the herds after they were 5 years old. The shortest period of fertile service was 1 year and 7 months, while the longest period of fertile service was 11 years and 1 month.

Forty per cent of the 2,982 services to fertile cows resulted in 1,197 conceptions, which is a ratio of 2.49 services.

Great variation in fertility was exhibited by individual sires as evidenced by a low fertility of 21 per cent by one sire and a high fertility of 71 per cent by another sire.

There was a decided but inconsistent decline in fertility of the sires as age advanced. At 5 to 7 years of age, the average fertility of the sires was

52 per cent and at 13 years of age or over it was 28 per cent. There was wide variation between individual sires.

On the average there was a decided trend toward lower fertility as the number of services per month increased, but the effect of the frequency of service in one month was most pronounced in the following month. When no services per month were permitted, the average fertility was 47 per cent the following month. When from 1 to 3 services per month were permitted the fertility for the following month dropped to 45 per cent, with a further consistent decline to 30 per cent when 10 or more services were permitted for the preceding month.

Season of the year apparently had little if any effect on fertility. Grouping the data from the standpoint of climatic conditions showed that the fertility was only 36 per cent for the sires used at the southern stations, whereas it was 49 per cent for the sires used at the western and northern stations.

Seventeen of the 20 sires averaged 41 per cent in fertility during the first 3 months following shipment to their respective stations and only 32 per cent after they had been at the stations 10 to 12 months. There was a general increase in average fertility until it reached a high of 47 per cent after 19 to 21 months at the station.

Because of the extreme and inconsistent variation in fertility exhibited by the individual sires on all phases included in this study, it is apparent that averages are of little value for application to individual sires.

**P42. Twelve Years with 1200 Cows. J. D. BRAGG, Agricultural Supervisor, Department of Public Welfare, State of Ohio.**

The Ohio Department of Public Welfare has the problems of housing, feeding, and clothing 36,000 state wards. These people must be guided morally and mentally while under its care in the twenty-three state institutions. Occupational therapy now furnishes numerous advantages to those who necessarily must be placed in the confines of the Ohio Welfare Institutions. The Welfare Department owns approximately 20,000 acres of land and cultivates about 12,000 acres in conjunction with the institutions. The dairy cow has become increasingly important to the members of the welfare family. She furnishes interesting and productive employment and a variety of wholesome and nutritious foods. The farming program is adjusted as nearly as possible to the needs of the dairy herds.

Twenty-seven hundred head of Holstein cattle located on sixteen farms furnish the bulk of the dairy products consumed and reproduce in surplus all of the heifers and bulls for replacements and for new herds. They give to those who care for them the pleasing task of working with something alive. These cattle have been responsive to kind treatment, careful feeding, selective breeding, sanitary housing, and sound health practices. As evidence, the herd average of more than twelve hundred cows has increased 49% in the last twelve years.

**P43. Artificial Insemination of Dairy Cattle.** C. L. COLE, University of Minnesota.

This experiment is being conducted in an attempt to test the practicability of artificial insemination in dairy herds under ordinary farm conditions.

The bull is kept at the North Central Station of the University of Minnesota and cows located from one to ten miles distant and in seven different herds are being artificially inseminated.

One herd of eighteen cows has had a very low breeding efficiency for the past several years and shows a rather low percentage for artificial insemination. It is, however, no lower than in previous years with natural breeding.

Another herd of twenty-three cows in which there has been continued trouble for several years with Bang's disease shows a very good breeding efficiency although it is impossible to make any comparison of records due to the 100% turnover of individuals during the past two years.

A third herd of forty that is disease free and on which complete breeding records have been kept for several years show a breeding efficiency considerably higher with artificial insemination than the preceding five year average.

The balance of the cows are located in small herds and their breeding histories are not available.

Sperm samples are collected by massaging the ampullae.

All the cows have been inseminated with fresh samples. The maximum age of any sperm used has not exceeded five hours.

The inseminating pipette is a glass tube about seventeen inches long,  $\frac{1}{4}$  inch outside diameter with a  $\frac{1}{16}$  inch bore. This is welded at right angles to a tube about five inches long with a  $\frac{3}{16}$  inch bore. It is advantageous to have a pocket blown in this piece to serve as a receptacle for the semen. A pyrex glass speculum  $1\frac{1}{4}$  inches in diameter and fourteen inches long and a flashlight complete the equipment.

The speculum is used to locate the cervix and a small quantity of semen is placed in the opening.

From available data, it seems that cows are best bred at the end of oestrus. We have made an effort to inseminate the majority at that time. Many of them have been inseminated from six to twenty-four hours after the passing oestrus with equally good results. Our data seems to indicate that better results will be obtained when cows are bred shortly after passing off heat than when bred at the first signs. Our data is not, however, complete or conclusive.

Three cows that had been both bred and inseminated several times and had failed to conceive were finally settled when small corpora lutea were removed from their ovaries during one oestrus and bred on the following oestrus. Another cow after failing to conceive was finally settled by rupturing the follicle mechanically and then inseminating her.

Extremely cold weather offers some obstacle since sudden chilling has a deleterious effect on the sperm.

Results seem to indicate that breeding efficiency can be maintained and often times improved by artificial insemination and that this method of breeding may offer an excellent opportunity to prove sires and greatly extend the use of already proven sires.

#### **P44. Relation Between Rate of Growth and Milk and Fat Production.**

H. P. DAVIS AND E. L. WILLETT, University of Nebraska.

There is an urgent need of means of predicting producing ability in dairy cows before a lactation has been completed. Prentice, in unpublished data, has indicated a correlation between feed consumption of calves and subsequent milk and fat production. Turner, in unpublished data, has hinted at a close correlation between pituitary activity and production. Seventy-six closely related Holstein females from the University of Nebraska herd were studied in regard to their rate of growth between birth and two years as correlated with their subsequent milk and fat production for the first and for their lifetime average of lactations corrected to 365 days, class B, maturity. The three measurements were weight, height at withers, and chest girth. The 76 females were compared to the standards established at the University of Nebraska and found to compare closely with them.

The group of 76 females were arranged according to percentage of increase in weight at two years over birth, in groups with 50 per cent class intervals, varying from 900-949.9 to 1750-1799.99, with an average of 1220.6. This latter figure represents the relationship between 87 pounds at birth and 1149 pounds at two years of age. The subsequent production for the first and lifetime lactations of each group is presented in a table. The same animals were arranged according to percentage increase in height at withers by two percent class intervals and ranged from 70-71.9 to 100-101.9 with production for these gains shown on the table. The same animals were also arranged according to percentage increase in chest girth by four per cent class intervals, ranging from 120-123.9 to 172-175.9.

Seventy-six Holstein females of the University of Nebraska herd, apparently normal according to standards established there, when their milk and fat records for the first and for the average of their lifetime lactations were arranged in tables according to the percentage rate of gain in weight, percentage increase in height at withers and percentage increase in chest girth, showed no significant relationship between rate of growth and production.

## EXTENSION SECTION

**E1. Methods of Presenting Different Extension Practices in the Testing Project.** GLEN W. VERGERONT, Wisconsin College of Agriculture.**I. THE PROBLEM. LET US TAKE INVENTORY.**

A. Our project meets with considerable competition with others for the time of the county agricultural agent.

B. Too many dairy herds have a level of production far below the line of profit.

1. A study of butterfat sales by one county agent indicated a variety of incomes from dairy products at the same milk plants.

2. THE COUNTY AGENT became interested in herd improvement.

C. Methods used to interest his dairymen.

1. A comparison of the average fat production per cow of the county, the state and the nation.

2. New D.H.I. Association members were encouraged to estimate production expected from each cow in herd.

3. Reasons for low producing herds.

D. COUNTY-WIDE PLANS for dairy herd improvement.

1. Plan to increase the net income for the dairy farmer.

2. D.H.I.A. testing is a good dairy farm management practice.

**II. Promotion.**

A. General publicity arousing a desire for herd improvement.

B. Newspaper publicity prepared in cooperation with college agricultural journalism department.

C. Schools.

1. Breeding schools put on by the college men.

2. Assistance at schools put on by breed associations.

D. Tours, Cattle Exhibits, Picnics, 4H demonstration and judging contests.

**III. Maintenance.** The responsibility for maintenance for associations rests with the extension dairymen and the county agricultural agent.

**IV. SUPERVISION AND INSPECTION.**

A. Farm visits to officers and members.

B. Inspection and assistance to fieldman.

C. District conferences.

**E3. An Extension Program Coordinating Dairying, Crops, Farm Engineering, Farm Management and Forestry.** A. R. MERRILL, Connecticut State College.

An Extension program must be practical for the section where it is to be

used. The reasons for this program must be sound and the results obtained must tend towards making for a stabilized agriculture.

The day of individual program making is past. No one specialist is important unto himself alone. No Extension program should be put out until it has had careful study by all specialists who may in any way be associated with its development. Individual subject matter should be handled only by the specialist who is directly informed on that subject, but all subject matter should fit into the general plan and be sound in practice.

Such studies will result in better programs and will educate both specialist and farmer toward a more uniform understanding of our extension problems.

**E4. A Method Used to Illustrate the Fact that Higher Producing Cows Make Larger Returns for Roughages.** W. T. CRANDALL, Cornell University.

Some farms are best adapted to the growing of roughage crops for which there is normally no satisfactory direct cash market. Dairy cows are kept on many of these farms as a means of marketing these unsalable farm crops and returning cash for them. It is just as important for dairy farmers to be interested in keeping good cows that will pay well for the home grown feed, as it is for them to sell their milk to a satisfactory market.

In order to emphasize the relation of better producing cows to higher returns for roughages, any sufficiently large number of cows with dairy herd improvement association records is divided into four groups. The division is made on the basis of yearly butterfat production and the range of selection is as follows: Under 250 pounds, 250-349 pounds, 350-449 pounds, over 449 pounds. A chart that is built up step by step during the progress of a lecture is used to show for each group the gross income, the costs of production other than for feed, the cost of grain and the amount remaining to pay for the pasture, silage and hay consumed. In the final addition to the build-up chart the amounts returned by the various groups for each ton of hay equivalent consumed (hay plus  $\frac{1}{3}$  silage) is shown. It is possible to make up this kind of material when complete yearly feed and production records are available on as few as 400 cows.

A discussion of the results obtained from a recent study of the records of 436 full time cows in one New York dairy herd improvement association will indicate the type of material that can be presented by this method. The average yearly butterfat production in pounds of each group was 210, 296, 387 and 477 respectively. The calculated costs other than feed, the grain cost and the charge for pasture were deducted from the gross income of each group and amount remaining was credited to the hay and silage consumed. The 210 pound group was credited with nothing for hay and silage, the 296 pound group with \$42, the 387 pound group with \$60 and the 477 pound group with \$82. On the basis of hay equivalent actually consumed the

groups paid the following amounts per ton: the 210 pound group, \$0.00; the 296 pound group, \$12.03; the 387 pound group, \$18.50; the 477 pound group, \$24.00.

**E5. An Extension Program in Grassland Farming.** C. B. BENDER, N. J. Agricultural Experiment Station.

The interest in a program of this type is stimulated by the same methods that are used to sell many other extension programs.

As this program includes both intensive pasture management and molasses grass silage as fundamental parts, the attack will vary with the season of the year. It is not necessary to start the program at any particular time of the year, the only essential is that the leader of the program should be enthusiastic about it because of the end results.

If the program is to be initiated on a state wide basis in the winter, meetings should be held in the various dairy centers of the state. At this time through talks and lantern slides the methods of carrying out the work, experimental results and economic experiences are brought out.

If any dairymen in the state have put up grass silage, barn meetings may be held at their farms at which time the leader of the project can discuss methods and objectives of the program and the dairyman can furnish his own experiences showing the practical feeding results he has obtained.

These meetings are followed up by news stories in the local and state press, circular letters sent out by the county agents and farm journal articles bringing experimental facts to light and submitting results that have been accomplished on dairy farms within the state.

In the spring and summer months field meetings should be held on pastures to explain this method of grassland management. Again it is extremely advisable to have the owner give the results he has obtained by following this program.

Barn and field meetings should be held while the silos are being filled with molasses grass silage. The dairymen may then have an opportunity to study the harvesting methods and the methods of adding the preservative. At this time yield and cost data may be stressed to give comparisons with corn silage. Furthermore comparative acre yields of protein should be stressed between legumes, grasses and corn. Increased use of silos as well as silo capacity figures are of value at these meetings.

The fact should be stressed at every opportunity that this is a program of erosion control which can be carried out by a farmer without embracing contour farming or strip farming methods. Molasses preservation of legumes and grasses is excellent crop insurance because it eliminates the hay making losses due to inclement weather. The method provides a better labor distribution and any dairy farmer who has a silo, silage and hay machinery can carry out the program successfully and with sound feeding economy.

**E6. A Method for Preventing Onion Flavor in Milk.** C. E. WYLLIE,  
University of Tennessee.

For over 150 years wild garlic or wild onions have gradually spread from the Atlantic coast to all sections of the country. While it affects several agricultural products it is becoming a serious menace to dairying in certain sections. It affects the milk and it is crowding out good pasture grasses. The methods which have been recognized for control of the onion problem are as follows: (1) Cultural methods, (2) Feeding methods, and (3) Removal of the flavor from milk or cream. Each of these methods has its peculiar advantages and adaptability.

**PREVENTION BY MOWING PASTURES**

It has been found that allyl sulphide in the onion tops is the cause of the onion flavor in milk. This unites with the butterfat. It is a volatile substance which passes off in the air when the tops are cut. The onions grow readily early in the spring ahead of other vegetation because a temperature of 30°-50° F. is favorable for their growth. The tops stop growing late in the spring.

In order to determine when to cut the tops, small plots, about eight feet square, are marked off for trial cuttings. A hoe is used to cut off the tops. If, on examination, a few days later, no new onions have come up it is time to mow the entire pasture with a mowing machine. When the onions are wilted, by the second day, cows may be pastured on such pasture for the summer season. This method is not satisfactory for handling the crop of onions which come up in the fall. Neither will this method eradicate the onion plant. This method of mowing pastures has been successfully used for more than ten years at the University of Tennessee in a pasture badly infested with onions.

**E7. The Use of Electric Fence in Bull Pen Construction.** JAS. W. LINN.

The customary bull pen is not adequate for the average breeder or dairyman.

In bull pen construction, the electric fence has its main value as a supplement to other fences and when properly installed with an efficient transformer, is safe and effective.

**E8. Dairy Cattle Breeding Schools.** E. E. HEIZER, Ohio State University.

The following outline presents subject matter covered in a two session breeding school. Film strips, motion pictures, charts and Herediscope are utilized as methods of presentation.

**FIRST SESSION**

***Principles of Heredity***

**(a) Biological Foundation.**

- The Cell—Ordinary cell division, formation of germ cells, control of sex.
- (b) Theories of Inheritance.
- Older Theories.
- The contribution of Mendel.
- One, two and three factor inheritance.
- Multiple factor inheritance.
- (c) Abnormal and lethal factors.
- (d) Variation and Selection.
- (e) Breeding Practices.
- Crossbreeding, Outcrossing, Linebreeding and Inbreeding.

## SECOND SESSION

*The Requirements of a Constructive Breeding Program*

1. Sound Health Program.  
Only healthy, disease-free cows produce efficiently.
2. A Complete Testing Program.  
Continuous testing of all cows in the herd.  
(a) Importance of environmental influences on production.
3. Intelligent Use of Records.  
(a) Cull inefficient cows.  
(b) Measure hereditary constitution of sires and breeding cows.  
(c) Interpretation of Proved Sire Records.  
(d) "Nicking" or Heterosis demonstrated by sire analysis.  
(e) Utilization of sires—cooperation, artificial breeding.  
(f) Importance of life span on profitability of dairy cows.  
(g) Cow Family Studies.  
(h) Herd Analysis.
4. Young Sire Selection.
5. A Breeder's Ideal.

**E13. Report of Quality Committee, Extension Section, American Dairy Science Association.** C. J. BABCOCK, Chairman, Bureau of Dairy Industry, United States Department of Agriculture.

A questionnaire sent to each of the States and returned by 46 of them revealed the following: Approximately 15.2 percent of our dairy extension is related to quality-improvement work. If evenly distributed and based on properly outlined projects for improving the quality of milk and cream as delivered to the plant, this percentage would not be unduly out of proportion. However, at least one-half of this extension work deals with methods of manufacturing dairy products. This lowers the percentage of extension work related to farm conditions to less than 9 percent. Looking at it from another angle; the 46 States reporting had on an average 1.7 men

working on dairy production compared with not over 0.15 of a man reported as doing work related to sanitary milk production, a proportion better than 10 to 1 for production over quality. Although this ratio is entirely too low and does not balance the importance of quality and production, it is actually too favorable to quality improvement. The ratio is correct according to the figures given, but a study of the projects reported reveals a large percentage of time has been credited to quality improvement that has no direct relation to improving the quality of milk and cream as delivered to the plant.

The questionnaire plainly revealed that a great majority of our States do not have a well defined or well outlined project for improving the quality of milk and cream.

Many of the States measure the results of quality work by what may be termed hearsay evidence. They have no established base from which to measure results. Neither do they have definite means for such measurements. This is largely due, no doubt, to the fact that they do not have well defined programs.

If the extension service would assume the leadership in a quality-improvement project, it could easily obtain the cooperation of the various agencies within the State necessary for its success.

The new projects which are to be started during the next year are few in number and, as a whole, show but little promise of accomplishing definite results.

Although practically all of the States admitted that insufficient extension work was being done on quality improvement, practically nothing is being done by our extension services to remedy this condition.

**E14. Progress Report of Quality Committee of Dairy Products of the Dairy Manufacturing Section of the American Dairy Science Association. W. H. E. REID, Missouri Agricultural Experiment Station.**

The quality of milk and milk products from the consumers point of view has received more than usual study during the past three years. Interest in this study has been manifested by our experiment stations and the different phases of the commercial industry. This interest includes all divisions of the dairy industry, production and manufacturing.

Consideration has been given to projects applicable on dairy farms and in dairy manufacturing plants which have for their objective improvement of methods practiced and equipment used in producing milk and the manufacture of dairy products which include butter, cheese, ice cream, condensed milk, evaporated milk and dry skim milk. These projects are broad in their scope so as to be applicable in all states where milk production is of primary importance.

It is obvious that the objectives of quality programs projected by production and manufacturing interests are quite alike and should be coordinated in order to effect maximum accomplishments. Progress should be greatly facilitated by acquiring a mutual understanding of programs of the different interests involved.

**E15. Milk Schools as a Means of Improving the Milk Supply.** J. A. NELSON, Montana State College.

Regular milk samples are collected by the milk inspector unbeknown to the producer. The samples are scored in accordance with the U.S.D.A. score card B.D.I. 64. A meeting of all the producers is called at which time the dairy extension specialist discusses the factors that make for a good milk. Samples with defects in flavor are used for demonstration and the possible remedies explained. The milk samples are listed according to quality and at the end of each meeting the producers are confidentially given their scores with the defects and possible causes explained. This method is used not only for market milk but also for milk which is to be manufactured into other dairy products.

**E18. What We Can Learn from Denmark in the Use of Artificial Insemination.** E. J. PERRY, New Jersey Agricultural College.

A close range study of cooperative artificial breeding in Denmark has revealed its success. During the past 21 months, 1,200 cows owned by 220 cooperating Danish farmers were inseminated by using the semen of two sires. The work is being done by a salaried veterinarian. His primary job is insemination. The balance of his time is divided between preconception treatment and examination for pregnancy both of which are performed when his regular duty brings him to the farm or neighborhood. The plan puts breeding on a scientific basis and is of particular value to the rank and file of dairy farmers who cannot afford to pay similar services individually. Every sample of semen is examined under the microscope before it is used. To date the artificial method in Denmark has required an average of 1.2 services per conception whereas the natural method has required 1.8 services, or a difference of  $33\frac{1}{3}$  per cent. Other recognized advantages are: 1. The blood of superior sires can be widely utilized at a very low cost per service. 2. Practically eliminates the spread of disease through service. 3. Eliminates the necessity of keeping and feeding a bull on each farm. 4. A large family of cattle is quickly established in a district.

The technique of collecting, preserving and using the semen should soon become fairly well standardized. The artificial vagina as a means of collection is in favor in Denmark, the British Isles, Holland, Russia. It is of particular advantage before bulls have reached an old age. By it the semen can be gathered quickly and probably in a little cleaner condition than by

other methods. Collection by massaging the accessory genital organs, the system developed in this country, is of particular advantage when using older bulls. While all bulls do not respond equally well to this method and the quantity of semen obtained is sometimes small, it nevertheless is proving fairly satisfactory in cases where the proper technique of massage has been learned. In large scale artificial breeding it is recommended that both of these systems of collection be mastered so that either can be practiced, as the circumstances require.

In a well organized artificial breeding project, which does not operate over too large a district, the yearly cost per cow should not exceed \$5.00. This figure is hardly as high as the yearly cost of bull service per cow in well managed herds where complete data including feed, labor, depreciation, housing and other items are included.

### MANUFACTURING SECTION

#### **M1. Some Factors Affecting the Estimation of Fat in Milk by the Babcock Method. W. A. CALDWELL AND E. O. HERREID, Vermont Agricultural Experiment Station.**

Several Vermont Dairy Plants have shown, according to their books, excessive losses of butterfat; that is they have been unable to account for the fat purchased when checked against the fat sold in milk and milk products. These so-called losses caused so much concern that plant managers brought this problem to the dairy department at the University of Vermont for assistance.

To determine the amount of fat unaccounted for, the losses in one plant were summarized by months over a period of four years. The years 1932, 1933, and 1934 showed a greater amount of fat unaccounted for during the first as compared to the last six months of each year. The same relationship existed in 1935 but the difference was small. In two small receiving stations handling only milk and cream, the intake was checked with the outgoing fat. At plant A on three consecutive days the fat unaccounted for was 5.10, 6.25 and 3.75 per cent respectively and at plant B the results showed a loss of 4.52 and 6.79 per cent on two consecutive days.

In view of the fact that difficulties were experienced in accounting for fat under conditions existing in these dairy plants, it was deemed advisable to approach this problem through a study of some of the factors that might affect the estimation of fat by the Babcock method. The factors studied were temperature of sampling milk, temperature of adding water, temperature of water bath, temperature and speed of centrifuge, and effect of reading the tests with glymol. Mojonnier fat determinations were made on the same samples.

Milk was sampled at temperatures of 40, 70, 100 and 140° F., and the results showed that within the extremes of 40 to 140° F., the test was decreased

by 0.08 per cent as the temperature was increased. Within a range of 40 to 100° F. the test was decreased by 0.04 per cent.

The influence of temperature of centrifuge was studied at 75, 140 and 180° F. and ten samples of milk averaged 4.21, 4.23 and 4.24 per cents respectively. With respect to speed, the centrifuge was operated at 850, 1050 and 1400 R. P. M. and the results averaged 3.94, 3.95 and 3.99 per cents respectively.

The temperatures of water bath studied were 100, 140 and 160° F. The average results were 4.33, 4.37 and 4.40 per cents respectively.

The last 980 tests made in the above experiments were read with and without glymol and the results showed that glymol reduced the average test by 0.165 per cent. Sixty samples of milk were tested in quadruplicate by the Babcock method and in duplicate by the Mojonnier method. The former were read from the bottom of the lower meniscus to the extreme top of the upper meniscus and by eliminating the upper meniscus with glymol. The average readings obtained were as follows: Mojonnier, 3.84 per cent; Babcock, read without glymol, 4.00 per cent and the Babcock read with glymol, 3.83 per cent.

All Babcock tests made in this study were run according to Vermont regulations and in addition a column-meter and a magnifying glass were used to read the tests to the nearest 0.05 and nearest 0.1 per cent. The results reported are for fresh milk.

## **M2. The Cause and Prevention of the Decrease in Fat Test of Composite Samples. R. F. HOLLAND, Cornell University.**

It has been known since 1890 that the Babcock tests made on composite fall about 0.1 per cent below the average as determined by daily tests on fresh milk.

The decline in test is most rapid during the first few days of storage and is greater when the samples are stored in composite sample bottles than when pipetted and stored in Babcock test bottles. The decline in test takes place when either the Mojonnier or the Babcock method of fat estimation is used.

The factors responsible for faulty dispersion of the fat such as "oiling off," churning, and packing of fat globules contribute most to the decline.

Saponin prevents the decline in test and can be used in samples to be tested by either the Babcock or Mojonnier methods. Five-tenths of a gram is sufficient for a 250 ml. sample.

Saponin reduces the danger of churning samples when preparing them for the test, and permits dispersion of the fat without the necessity of heating the sample.

## **M3. A Study of the Resazurin Test as Applied to Cream. HERBERT JENKINS, New England Dairies, Inc., Boston, Mass.**

Shortly after the publication of Ramsdell, Johnson and Evans' report on resazurin as an indicator of the sanitary condition of milk, experimental work was started in these laboratories to determine whether or not resazurin could be used for the same purpose with pasteurized cream.

The research program was threefold: first, to ascertain the proper concentration of resazurin dye to use; second, to set a standard on the basis of reduction time for good, fair, and poor quality cream; third, to find out if the resazurin test would be a more rapid indicator of the quality of cream than the Methylene Blue Test. More than four thousand samples of cream of varying quality were used in this study which covered an entire year. Comparisons were made with the following tests: resazurin, methylene blue, microscopic, standard plate, and acidity.

It was found that one-tenth of one cc of .05 per cent resazurin dye solution is the proper amount to use with ten cc of cream. Procedure is then the same as for the Methylene Blue Test. The cream-resazurin mixture is grey in color and reduction time is recorded when the grey turns to pink. Creams reducing in less than one hour were generally found to have poor keeping qualities and had bacteria counts above 300,000 per cc. Creams which did not reduce in less than two hours had bacteria counts below 100,000 per cc. If pasteurized cream did not reduce within three hours with the resazurin test, it could be relied upon to have good keeping quality and to have a low bacteria count. A two-hour incubation period with the resazurin test gives results comparable to five hours with the Methylene Blue Test. Cream which reduced resazurin in five to fifteen minutes takes from one to one and one-half hours to reduce methylene blue. Results indicate that the resazurin test is a practical test for determining the bacterial quality of pasteurized cream and is superior to the Methylene Blue Test in that results are obtained more rapidly.

#### **M4. Studies of Lipase Action in Milk. VLADIMIR N. KRUKOVSKY AND B. L. HERRINGTON, Cornell University.**

The extent of lipolysis of the fat in milk was determined by titrating the free fatty acids in butter-oil prepared from the milk. It was possible to show that lipolysis occurs in normal mixed milk between the time of milking and the time of delivery at the pasteurizing plant.

A study of the milk of individual cows indicated the presence of active lipase in practically all samples, though there is considerable individual variation.

A study of commercial pasteurized milk sold in the State of New York revealed much larger quantities of free fatty acids than are normally present in fresh milk.

By warming cold raw milk to approximately 30° C., and then cooling slowly, the subsequent rate of lipolysis was greatly increased.

Evidence was obtained showing that milk contains at least two lipases. One is very sensitive to formaldehyde. The other is quite resistant to it. The relative amounts of these two enzymes varies in different samples of milk.

**M5. Observations on the Lipase Activity in Cow's Milk.** J. C. PFEFFER, H. C. JACKSON AND K. G. WECKEL, University of Wisconsin.

This investigation deals with a study of the lipase activity of cow's milk. The method of Nair was employed in all experiments to determine the lipase activity.

The authors found that no direct correlation existed between milk of late lactation and lipase activity. A direct relationship, however, was found between the amount of milk produced and such activity. Less lipolytic activity was observed when the milkings were ten pounds or less than when the milkings exceeded fifteen pounds.

The lipase activity of the milk from an individual cow does not vary to any great extent from day to day.

A marked decrease in lipase activity of milk was observed when cows are taken from pasture and placed on dry feed. This decrease in activity is not permanent, as it returns to normal within two weeks. A similar decrease is observed when cows are changed from dry feed to pasture.

The lipolytic factor is carried in the serum of the milk. When milk is separated, greater lipolytic activity is observed in the skimmilk than in the cream. As the fat content of the cream is increased the lipolytic activity decreases.

An increase in temperature of the milk during separation causes a decrease of lipase activity of both the skimmilk and cream. A marked decrease occurs when the milk is separated at 120° F. The slime of the separator bowl is an excellent source of lipase and possesses approximately three times the lipolytic activity of the original milk. The decreased activity resulting from higher temperatures of separation is believed due to inhibition of the enzyme by heat. Since the inhibition is approximately the same in cream, skimmilk and separator slime, it appears the effect of higher temperatures of separation is due to inactivation rather than fractionation of the enzyme.

The increased activity of lipase after homogenization of raw milk is not due entirely to decrease in fat globule size. A very slight difference is observed when untreated milk is added to homogenized and unhomogenized substrates.

Salt has an inhibiting effect on the enzyme. Salted raw cream butter develops less acidity than raw unsalted butter.

**M6. Detecting Milk that May Become Oxidized.** GEORGE R. GREENBANK, Bureau of Dairy Industry, U. S. D. A.

A simple method of determining the susceptibility of milk to oxidized flavor has been developed. A small amount of copper sulfate is added to the milk and the increase in oxidation-reduction potential determined. Unusual increases in potential indicate samples that may become oxidized. From 3 to 6 hours are required to complete the test. On samples of known origin and treatment the test has been at least 90 per cent reliable.

**M7. The Relation of Oxidation-Reduction Potential to Oxidized Flavor in Milk.** GEORGE R. GREENBANK, Bureau of Dairy Industry, U. S. D. A.

A study was made of the nature of the oxidized flavor and its relation to the oxidation-reduction potential of the milk.

The conclusions are based on examination of more than 3,000 samples of milk from many cows.

Thermal inhibition of the flavor is shown to act through a lowering of the oxidation-reduction potential.

The effect of metallic contamination on the flavor is shown to vary with the metal and its physico-chemical state.

Inhibition of the flavor by a change from dry to green feed is paralleled by a decrease in oxidation-reduction potential and an increase in poisoning action.

Light may inhibit, promote, or have no effect on the development of the flavor, depending on contamination and intensity of irradiation.

A schematic equation is presented which indicates the nature of the oxidation. A theory in keeping with the observations made during this study is presented to describe the nature of the oxidation. The equation assumes a mild chemical oxidation.

Many isolated facts presented by other workers are explained by means of oxidation-reduction potential changes.

**M8. A Study of the Relation of Titratable Acidity to Metal-Developed Oxidized Flavor in Milk.** W. CARSON BROWN AND R. B. DUSTMAN, West Virginia Experiment Station.

At the West Virginia Experiment Station a study of the acidity of 220 individual samples of freshly drawn cow's milk has shown no apparent correlation between the natural acidity of the milk and its tendency to develop oxidized flavor when contaminated with copper. All experiments in which acidity was studied were carried out on pasteurized winter milk and the tendency to develop oxidized flavor was determined by the addition of 1.3 p.p.m. of copper added after pasteurization, followed by a three-day storage period of 35° to 40° F. The presence of oxidized flavor was determined by taste.

Anderson, Dowd, and Stuewer (1937) found that the titratable acidity of winter milk is higher than that of summer milk. Since this seasonal change occurs coincidental with the change of susceptibility of milk to oxidized flavor, it seemed possible that this condition may have been partially responsible for oxidized flavor. Anderson and his co-workers found an association between acidity and the tendency to develop oxidized flavor and that "milk of high acidity invariably developed an oxidized flavor upon pasteurization." However, at West Virginia, the analysis of the data obtained from 220 individual cows' samples did not show any difference in the apparent acidity of the milk from cows whose milks were susceptible to oxidized flavor and those whose milks were free from this defect.

Anderson and co-workers were able to eliminate oxidized flavor from a commercial milk supply by reducing the titratable acidity to 0.145 per cent. However, in the present study neutralization to 0.13 per cent titratable acidity, or in a small number of cases to 0.10 per cent, did not effect the development of the flavor.

No explanation is offered for the discrepancy in results which have occurred except that possibly the milk used by Anderson and his co-workers was subject to the development of oxidized flavor without copper contamination while the milk used in these trials would not develop oxidized flavor unless contaminated with copper. It is also possible that in their work, because of neutralization, the milk did not dissolve sufficient copper from the equipment to cause the flavor to develop. In all trials herein reported the copper was added after pasteurization in the form of a copper sulphate solution. In view of these facts it would have been desirable to have made several trials upon naturally susceptible milk, but during the past two years milk of this type has not been available, except in rare cases, in the University herd.

The results of these experiments indicate that metal-developed oxidized flavor is not related to the acidity of freshly drawn milk and that the standardization of the titratable acidity to 0.13 per cent does not decrease the tendency of oxidized flavor to develop.

**M9. Studies on the Activated Flavor of Milk. J. C. FLAKE, H. C. JACKSON AND K. G. WECKEL, University of Wisconsin.**

The specific flavor defect which is sometimes apparent in over-irradiated commercial vitamin D milk, and known as activated flavor may best be described by the terms burnt protein or burnt feathers. The terms mushroom and sunshine flavor are sometimes used. The flavor is greatly intensified by heating the irradiated milk to 180° F. or higher.

Studies were instigated to determine the conditions under which the flavor develops and something of the chemical characteristics of the compound responsible for the flavor.

It was found that when milk was heated to temperatures above 150° to 160° F. and cooled, previous to irradiation, the resulting irradiated milk had a more intense flavor than when either raw or ordinary pasteurized milk was irradiated.

Attempts at altering the salt balance of the milk by addition of sodium citrate, di-sodium phosphate, calcium acetate, calcium chloride, calcium lactate, or di-basic calcium phosphate failed to increase or decrease the flavor intensity.

It was found possible to remove the activated flavor from milk by oxidation. The addition of a small amount of either hydrogen or calcium peroxide either before or after irradiation caused a marked decrease in intensity of flavor. It was possible to remove the flavor by adding two to three parts per million of copper to the irradiated milk followed by bubbling air through the milk either at room or higher temperature. The best results with this method were secured by holding the milk at 140–145° F. for 30 minutes and bubbling air through it during this interval. Oxidized flavor eventually developed in the milk given this second treatment, when it was cooled and held at 40° F., but only after 36 to 48 hours. However, irradiated milk whether given this treatment or handled normally shows no greater tendency for development of oxidized flavor than does normal milk.

The studies indicated that when irradiation and homogenization are both used in processing milk better results are secured if irradiation precedes homogenization.

#### **M10. Variation in the Composition of Milk and the Effect on Solids-not-Fat. H. A. HERMAN, University of Missouri.\***

Variations in the fat and solids-not-fat content of milk are often the contributing factors for controversies, many times of legal nature, in the selling and buying of milk. In an effort to gain additional information concerning the extent and nature of these variations the milk produced by the Missouri Station herd is being analyzed daily for fat and total solids content. In addition the milk from 25 cows is being analyzed every other week for total nitrogen, lactose, and chlorides. The effects of feeding, stage of lactation, season and temperature, age of the cow, and under soundness on the composition of the milk are included as a part of this study.

The data gathered to date indicate that season or temperature, it being very difficult to separate these two factors under practical conditions, largely accounts for the decline in the solids-not-fat content of the milk produced during the summer months.

In the herd of 60 cows, made up of 49 percent Holsteins, 39 percent Jerseys, and 12 percent Guernseys, the solids-not-fat content of mixed herd milk

\* Contribution from the Department of Dairy Husbandry, Missouri Agr. Exp. Sta., Journal Series No. 563.

for the past twelve months has been observed to range from 8.0 to 9.7 percent. During July, August, and September of 1937, however, the range was from 8.1 to 8.7 percent, with an average solids-not-fat value of 8.45 percent. The majority of samples were above the legal standard of 8.25 percent solids-not-fat, but during July and August in particular, nearly 50 percent of the samples gave readings below 8.5 percent, the standard set by some cities. The temperature during these two months was seldom above 95° F., and was hardly comparable to the drought years of 1934-35-36 when the mercury often reached 110° F. The cows were fed largely on hay, silage, and grain, with limited blue grass pasture.

Turning the cows on succulent barley pasture in April and May resulted in an increased milk yield, but so far as can be determined the solids-not-fat content of the milk was not affected, averaging 8.4 to 9.5 percent for this period. Analyses of the milk produced during the summer months, when the solids-not-fat was lowest, showed the lactose and totals nitrogen to be lower than previous, and the chloride content increased. During the fall and winter months the total nitrogen and lactose increased. The chloride content of the milk increased gradually throughout the lactation period.

The solids-not-fat content of the milk of individual cows was found to be high immediately following freshening, but to decline during the height of milk flow, and increase as lactation progresses. The lactose content of the milk decreased quite markedly near the end of lactation and the total nitrogen and chloride content increased. The increase in chloride content near the end of lactation is quite sharp and it seems apparent that the osmotic pressure of the milk is maintained at this period by the substitution of ionized chloride for lactose.

**M11. Studies on the Mold Mycelia Content of Sour Cream Butter. J. ADAMS AND E. H. PARFITT, Purdue University.**

Examinations have been made on commercial samples of butter manufactured between August 1 and April 1, using the technique suggested by Wildman for the examination of mold mycelia in butter. In addition, controlled samples have been prepared to determine factors influencing the amount of mycelia in butter.

A definite seasonal trend was found in the mycelia content of commercial butter, being highest during the summer months and lowest during the winter months. In a study involving 205 samples of commercial butter made during December and January, 99 per cent of the 103 samples which were graded as first grade had mycelia counts of less than 40 per cent positive fields, and 41.1 per cent of the 102 samples which were second grade had mycelia counts of less than 40 per cent positive fields.

The retention of mold mycelia in butter was found to vary from 30 to 60 per cent of the total mold content of the cream and such factors as wash-

ing, working of the butter, and pH of cream at the time of churning had no appreciable effect upon the amount of mold mycelia retained in the butter. Factors influencing the growth of mold in cream such as age, incubation temperature, amount of cream surface exposed to air, and agitation of cream during holding have been studied and found to affect mold mycelia count of the butter. A method has been developed using a fat solvent for determining the mold mycelia in cream.

**M12. The Effect of Temperature Upon Score Value and Physical Structure of Butter.** W. H. E. REID AND W. S. ARBUCKLE, University of Missouri.\*

Recent studies reveal that temperature is an important factor influencing the flavor of various dairy products. Submerged flavors exist at lower serving temperatures of the product, while full pronounced flavors are manifested at high serving temperatures.

When samples of butter were scored at temperatures of 40, 50, 60 and 70 degrees Fahrenheit, those samples at 50 degrees Fahrenheit received .5 to 2.5 points lower flavor score than at other temperatures. This procedure serves as a means of determining the quality of the cream used, efficiency of plant methods and the treatment butter receives subsequent to manufacture. It was observed that as the temperature of the butter was increased, flavors that are normally submerged at lower temperatures become apparent. The flavor score of butter manufactured from high quality cream was enhanced as the temperatures were raised, whereas the flavor score of butter made from cream of a questionable quality declined with increased temperatures.

Graphs showing rise in temperature of cubes, quarter and one pound prints of butter exposed at 80 degrees Fahrenheit indicated rapid temperature increase in the cubes, while a much slower rise was shown in quarter and pound prints.

A comparison of the structure and body consistency of the butters at different temperatures is being made by the use of the microscope.

**M13. Application of the Burri Smear Culture Technic to the Examination of Butter.** H. F. LONG AND B. W. HAMMER, Iowa Agricultural Experiment Station.

The Burri smear culture technic can be used to examine butter by picking small portions with a platinum needle, and culturing each on the surface of a dry agar slope. After incubation the colonies on the slopes are counted and studied for colony types. The maximum number of colonies on a slope that can be counted readily is about 100 although larger numbers can often

\* Contribution from the Department of Dairy Husbandry, Missouri Agr. Exp. Sta., Journal Series No. 556.

be estimated satisfactorily. Various media and incubation conditions can be used with the procedure. If a differential medium, such as one containing milk and fat for the detection of proteolytic and lipolytic organisms, respectively, is employed, it can be poured into a plate and each portion of the butter smeared over a segment of the plate.

Many normal and abnormal samples of both commercial and experimental butter have been examined with the technic, using beef infusion agar and an incubation of 4 or 5 days at 21° C; the portions of butter were picked under a low power binocular to keep the size as uniform as possible and averaged approximately 1/20,000 of a gram.

The results indicate that the distribution of bacteria in butter, as regards both numbers and colony types, is often highly irregular. Such a distribution emphasizes that the growth of bacteria in butter is largely limited to certain points, presumably infected moisture droplets. It may be influenced not only by the original contamination of the butter but also by other factors such as irregularities in salt distribution, etc. The plate method gives no information on the distribution of organisms in butter because of the size of the sample taken and mixing of the organisms in it during plating.

The Burri technic often gives lower total counts than the plate method, due probably to the failure to break up clumps of bacteria and to overcrowding on slopes; with heavily seeded slopes it is difficult to distinguish the individual colonies and some organisms may even fail to grow.

Since all of the colonies developing on the slopes are at the surface of the agar, a better differentiation into colony types is obtained with Burri technic than with plates in which some colonies are subsurface. Occasionally, colony types have been noted on Burri slopes which did not grow on plates or which were diluted out in the plates suitable for examination.

#### **M14. The Application of the Phosphatase Test to the Butter Industry.**

W. H. BROWN, E. H. PARFITT, Purdue University.

The phosphatase test as it is used for milk has been applied to butter to determine whether or not the cream has been properly pasteurized prior to churning. The Scharer technique, with one ml. of butter serum used to replace the one ml. of milk as the directions indicate, has been followed. The butter serum is collected by centrifuging the sample of melted butter.

The application of the phosphatase test on the butter serum has shown:

That of 372 samples of commercial butter analyzed, 31.2 per cent gave a positive phosphatase test. A larger percentage of the samples that gave a positive phosphatase test, when subjected to the keeping quality test of 15.4° C. for 10 days, dropped in score more than did those that gave a negative phosphatase test.

The butter serum tended to give a more positive reaction than did the corresponding cream and there exists the possibility of the manufacturing process of the butter influencing the phosphatase reaction.

**M15. Preliminary Studies of the Neutralization of Cream for Butter-making.** R. C. TOWNLEY AND I. A. GOULD, Michigan State College.

In an effort to determine the efficiency of various neutralizers for sour cream and to ascertain the accuracy with which they reduce the acidity of the cream to different calculated acidity ranges, seven neutralizing agents were studied. These neutralizers were sodium carbonate, sodium bicarbonate, a mixed sodium carbonate-sodium bicarbonate neutralizer, a newly recommended commercial product which on the basis of analysis is apparently composed chiefly of sodium and potassium carbonates, sodium hydroxide, magnesium lime, and calcium hydrate.

The original titratable acidity of the cream used throughout this study was approximately 0.5 per cent, expressed as lactic acid. This cream showed a pH of about 4.7 and a fat content of 30 to 35 per cent. The neutralizing factors used were those submitted by the manufacturers for the commercial products, or, in the case of the other products, were those which were found to be correct by the neutralization of lactic acid. The additions of the neutralizers were made on the basis of calculations to reduce the acidity of the cream to the following percentages: 0.25, 0.15, 0.10, and 0.05.

The preliminary findings indicate wide variations in the efficiency of acid reduction by these neutralizers. In general, the carbonate neutralizers failed to reduce the acidity to the calculated point with the reduction being only slightly in error at 0.25 per cent, but being more widely in error when efforts were made to reduce the acidity to a lower range. The ranges of the values secured by the different carbonate neutralizers which correspond to the theoretically expected acidities of 0.25, 0.15, 0.10, and 0.05 per cent, were 0.26 to 0.29 per cent, 0.19 to 0.23 per cent, 0.145 to 0.18 per cent, and 0.13 to 0.165 per cent, respectively.

The limes gave more accurate reductions than the carbonates throughout the acid range studied, with the values being close to those theoretically expected at acidities of 0.25 per cent and 0.15 per cent, but tending to be somewhat higher at the lower acidities. The caustic soda gave the most accurate reduction of any of the neutralizers studied with the values corresponding to 0.25, 0.15, 0.10, and 0.05 per cent, being approximately 0.23, 0.15, 0.11, and 0.075 per cent.

The pH determinations, made before and following the processing, showed variations to occur when different neutralizers were used. When the neutralizers were added at a rate calculated to reduce the acidity of the cream to 0.05 per cent, the average pH values of the cream were as follows: sodium carbonate, pH 6.7; sodium bicarbonate, pH 6.82; mixed soda neutralizer, pH 7.13; mixed sodium and potassium carbonate neutralizer, pH 6.82; sodium hydroxide, pH 7.42; magnesium lime, pH 7.16; and calcium hydrate, pH 7.01.

- M16. The Relation of Milk Quality to Grade of Swiss Cheese.** L. A. ROGERS, ROBERT E. HARDELL, AND FRED FEUTZ, Bureau of Dairy Industry, U. S. D. A., in cooperation with the Ohio State University and the University of Wisconsin.

While it is generally conceded that milk of low bacterial content is required for making Swiss cheese, no exact data have been available.

Data collected in 1936 and 1937, representing over 400 cheeses made in 20 factories in Ohio and Wisconsin, show that, in general, the number of first-grade cheeses increases directly with the quality of the milk as measured by the methylen-blue test. The effect of the bacteriological condition of the milk is also evident in the development of acid during the making process, which in turn has a definite relation to the grade of the cheese. If the milk is deficient in lactic bacteria so that the pH at dipping is not lower than 6.50 the chances are three to one that the cheese will be undergrade. If there are too many acid-forming bacteria capable of growing at the high temperatures maintained in the kettle and the pH at dipping is below 6.40, the chances of making a first-grade cheese are less than even. If the milk reduces methylene blue in less than 3 hours and the pH at dipping is less than 6.40 the chance of making a first-grade cheese is about one in three. If the methylene-blue reduction time is over 3 hours and the pH at dipping is between 6.40 and 6.50 the chances that the cheese will be first grade are about three in four.

- M17. Clarification of Milk for the Manufacture of Swiss Cheese, with Special Reference to the Use of Mastitis Milk.** KENNETH J. MATHIESON, GEORGE P. SANDERS, LLOYD A. BURKEY, AND J. FRANK CONE, Bureau of Dairy Industry, U. S. Department of Agriculture.

Data are presented dealing with the causes for the differences that exist between clarified and unclarified milk, together with the demonstrated value of clarifying mastitis milk used in the manufacture of Swiss cheese. The conclusions are as follows:

There is definitely more oxygen in clarified than in unclarified milk. Oxygen in milk may influence the set of the resulting cheese.

Clarification promotes the efficiency of the Swiss-cheese cultures as determined by pH measurements.

Both unripened and ripened unclarified-milk cheese contains more water than the clarified-milk cheese. Penetrometer readings indicate that the unclarified-milk cheese is softer in texture than the clarified-milk cheese.

The set of the cheese is increased with low as compared to high speed clarification and where low instead of high clarifying temperatures are used for the milk. The set of the cheese is increased, as compared to suitable checks, when the removed slime is returned to clarified milks, and when unclarified gravity cream is employed as a source of fat.

There is a correlation between the set of the cheese and the fat clusters. As the number of fat clusters is reduced the set of the cheese is improved.

Clarified-milk cheese usually contains slightly less lactose at 21 hours than unclarified-milk cheese.

The set of clarified-milk cheese is increased with an increase of the water content. It is also increased when there is an increase of the pH value at 21 hours.

With clarified mastitis milk the number of leucocytes is reduced about two-thirds as compared to the unclarified milk; and the rate of acid development is more rapid at the 8-hour period in the cheese.

The set of the cheese is increased as the number of leucocytes increases.

In 22 comparisons of clarified with unclarified mastitis milk the grades of the cheese were as follows:

Clarified mastitis milk cheese: Fancy, 4.54 per cent; Specials, 22.72 per cent; No. 1, 68.18 per cent; and No. 2, 4.54 per cent.

Unclarified mastitis milk cheese: Special, 9.09 per cent; No. 2, 90.90 per cent.

Clarification of mastitis milk is capable of causing the cheese made from such milk to be a Special, a No. 1, and even a Fancy, instead of a pin-eyed nissler.

There was only 1 typical pin-eyed nissler in the clarified-milk cheese, while there were 17 of this type in the unclarified-milk cheese.

It is to be understood that this question has been attacked solely as a research problem in manufacturing, and no implication is to be made regarding its possible public health significance.

#### **M18. Control of Types of Organisms in High Temperature Starters.**

DAVE NUSBAUM AND WALTER V. PRICE, University of Wisconsin.

It is difficult for cheese makers to carry pure cultures of *S. thermophilus* and *L. bulgaricus* under factory conditions. Some Swiss cheese makers desire a mixed culture of the two organisms but experience difficulty in maintaining them in a single starter in the correct proportions. Brick cheese makers using these organisms should probably use a pure or practically pure culture of one or the other. In this study an attempt has been made to propagate and maintain as desired either a mixture or a pure culture of these organisms.

A technique was adopted which can be employed by any factory not equipped with laboratory facilities. By even slight variations either in incubation period, size of inoculation or both, a cheese maker can carry a mixed starter of these two organisms in any desired proportion. Such proportions can be regulated accurately enough for all practical purposes by simply observing the taste and titratable acidity of the starter from day to day. It was found that when a typical mixed starter, consisting of approxi-

mately equal numbers of rods and cocci, is propagated with 5 per cent inoculations and 30 hour incubation periods, it can be changed in a four day period to a mixture containing about 99 rods to each coccus. By using lighter inoculations and shorter incubation periods the same starter in the same time can be made essentially a pure culture cocci. These procedures have been repeated with similar results using several different strains of *S. thermophilus*. All cultures were carried in the same incubator at 37° C. Photomicrographs of stained preparations of each starter have been prepared to show the change in proportion of organisms at the end of each incubation period when the various cultural practices were employed.

Wisconsin operators obtain pure cultures at weekly or bi-weekly intervals from the University Department of Bacteriology. Many of them inoculate each day's starter directly from these cultures until a new supply is delivered. Work is now in progress which shows the length of time that mixed and pure cultures of these starter organisms will remain viable and active when stored at temperatures of 4° C., 20° C., and 37° C.

**M19. Methods of Determining Chlorine in Milk and their Application in the Detection of Mastitis.** GEORGE P. SANDERS, Bureau of Dairy Industry, U. S. D. A.

Direct titrations of chlorine in milk with silver nitrate and either potassium chromate (Mohr's method) or dichlorofluorescein indicator yield results which are so erroneously high and erratic that the use of either method is not considered justifiable. Digestion by boiling samples in the presence of an excess of silver nitrate together with nitric acid and potassium permanganate (open Carius method), followed by titration with potassium thiocyanate in the presence of ferric alum indicator (Volhard titration), yields quantitatively accurate results.

The latter method has been so simplified, by combining reagents and omitting digestion, that it is both accurate and easy. The recommended procedure is as follows:

Ten cubic centimeters of milk is measured accurately and to it is added a measured quantity of the 0.0291 *N* special silver nitrate solution described below, more than sufficient to combine with all of the chlorine; ordinarily 15 cc. is the amount used. The mixture is stirred slightly and about 100 cc. of water is then added; it is titrated immediately with a 0.0291 *N* (2.828 grams in 1 liter) solution of potassium thiocyanate. The number of cc. of silver nitrate solution minus the titration value, multiplied by 0.01, equals the percentage of chlorine.

**Special silver nitrate solution:** Exactly 4.944 grams of silver nitrate is dissolved in water; 200 cc. of concentrated nitric acid and 300 cc. of a saturated solution of ferric ammonium sulfate (alum) indicator are added and the mixture is cooled and made up to 1 liter. The solution is standardized against the thiocyanate solution before use.

A study of data obtained in periodic tests in quarter-udder samples of milk from 29 cows, beginning in most cases with first-calf heifers and continuing through more than one lactation, shows that the determination of chlorine is one of the best of the several tests used for the detection of mastitis. Milks with chlorine values above 0.15 per cent were in all cases mastitis-positive; values between 0.12 and 0.15 indicated either suspected or definitely positive cases. Clinical symptoms varied so widely in different quarters of the udder at the same milking that it was found very essential to test quarter samples rather than all the milk of a milking. After the cow's recovery from mastitis, the chlorine value of the milk, like the yield of milk, usually failed to return to the normal level during the same or succeeding lactations.

**M20. Controlling the Fat Content of Swiss Cheese in Southern Wisconsin.** WALTER V. PRICE, University of Wisconsin.

From July 1936 to June 1937, four analysts measured the daily variations in composition of milk, curd, whey and cheese and recorded observations of the quality of milk and cheese at each of four Swiss cheese factories. These data were obtained from more than 3,800 kettles. The object of this work was to ascertain the feasibility of controlling the fat content of Swiss cheese by analytical and standardizing methods.

The factors responsible for variations in cheese composition are discussed in this report and the limits of accuracy of standardizing methods are indicated. It seems apparent from this study that, although the composition of the kettle milk can be controlled, the unpredictable variations in cheese composition make it impractical at present to attempt to guarantee a minimum of 45 per cent fat in the dry matter of every wheel of Swiss cheese as long as present commercial grades are observed.

**M21. Sodium per Borate as a Corrosion Inhibitor for Washing Powders.** LAWRENCE L. LITTLE, Meadow Gold Milk Plant, Oklahoma City.

A study was made of the effect of varying amounts of sodium per borate in preventing the corrosion of tin and aluminum in five per cent solutions of sodium meta silicate, trisodium phosphate, and sodium carbonate. Amounts of sodium per borate ranging from five to ten per cent were found to be effective in preventing the corrosion of both tin and aluminum in solutions of sodium meta silicate, while corresponding amounts had no effect in preventing the corrosion of tin and aluminum in five per cent solutions of trisodium phosphate or sodium carbonate. However, combinations of sodium meta silicate and sodium per borate were found to be effective in preventing corrosion of tin and aluminum in solutions of trisodium phosphate and sodium carbonate.

Trisodium phosphate is rendered non-corrosive to both tin and aluminum by the addition of ten per cent sodium per borate, provided at least twenty per cent sodium meta silicate is included in the trisodium phosphate portion.

Sodium carbonate is made non-corrosive to both tin and aluminum when five per cent sodium per borate is added, provided the sodium carbonate contains a minimum of ten per cent sodium meta silicate.

**M22. Sterilization by Irradiation—A Possible New Tool for the Dairy Industry.** O. F. GARRETT AND R. B. ARNOLD, New Jersey Agricultural Experiment Station.

Recent development of lamps whose radiations have high bactericidal power has led to practical applications of this principle in certain fields of endeavor. The practical sterilization of the surfaces of meats and bakery goods has been successfully accomplished. Installations in restaurants and at soda fountains for the sterilization of drinking glasses have been successful. A battery of these lamps installed above a hospital operating table has resulted in a great decrease in post-operative infections. The practical sterilization of the air of rooms is being studied.

To determine whether this type of death-ray had a possible practical application in the dairy industry a number of preliminary experiments at the New Jersey Station have been completed. Various utensils and containers such as large dippers, pails, ice cream cans, 10-gal. milk cans, and glass milk bottles were seeded with bacteria and then exposed to radiation for various times of exposures. At the end of 3 minutes the 2-quart dippers were completely sterilized, the straight-sided 5-gal. ice cream cans showed 99% destruction and the 10-gal. milk cans showed 95% destruction.

Exposure of a seeded spray vat pasteurizer and a coil vat pasteurizer showed very good bactericidal action of the radiations.

These preliminary results suggest the possibility of practical application of this type of sterilization to the control of bacterial growth in dairy utensils and equipment, to the control of outbreaks of thermophiles, ropy organisms, etc., in dairy plants, to the sterilization of paper and glass milk bottles, and perhaps as a help to the control of disease in the dairy herd.

**M23. Kefir Buttermilk.** LLOYD A. BURNEY, Bureau of Dairy Industry, U. S. D. A.

A method has been worked out for making buttermilk by the use of kefir grains. The method for the most part consists of immersing kefir grains in milk near the surface for 24 to 48 hours at a temperature of 65° to 70° F. by enclosing them in a cheesecloth bag or by some other porous container.

The advantages of this method of preparing buttermilk are that the culture remains active indefinitely without much danger of contamination, very little equipment or technique is required, and the product produced is highly flavored. The flavor can be varied at will by varying the temperature and

time of incubation and the method of using the grains. Such variations in conditions can be so controlled as to give buttermilk of alcoholic, of mildly acid, or of highly acid taste.

**M24. The Present Status of the Development of Fiber from Casein.**

**EARLE O. WHITTIER**, Bureau of Dairy Industry, U. S. D. A.

Casein fiber has been produced in Italy on a rapidly increasing scale for the past 2 years and production is now beginning in other countries. The fiber resembles wool, but may be produced of widely differing diameters, lengths, and physical characteristics. The casein required must be made under carefully controlled conditions such as already prevail in a considerable number of our domestic casein plants. One year's production of milk by an average cow (4,000 pounds) will furnish enough casein for 100 pounds of casein fiber.

**M25. Whey Solids in Candy.** **BYRON H. WEBB**, Bureau of Dairy Industry, U. S. D. A.

On the basis of experimental work in which whey solids have been incorporated in different types of candy, it appears probable that an important outlet for whey solids can be developed in the candy industry. Two of the three chief solid constituents of whey, lactose and protein, have been shown to be of distinct value in certain candies. Whey protein is of value in candy as an improver of body and flavor. In some candies in which whipped sweetened condensed whey is used, the whey protein is an important factor in foam production. Lactose in candy is essentially a substitute for sucrose and in such a rôle it pleasantly decreases the sweetness of the ordinary confection. The third whey solid, the salt, is of lesser importance and even limits the quantity of whey which may be used in certain candies.

Whey, chiefly in the form of sweetened condensed whey, has proved especially promising in the development of tentative formulas for caramels, fudge and toffee. Some progress has been made in the utilization of whipped sweetened condensed whey as a frappé for candy makers. Highly concentrated sweetened condensed whey with the lactose properly crystallized has been used as a major constituent of fondant. The usefulness of whey in fondant may be limited to colored goods because of its greenish yellow cast and tendency to darken with age.

It has been shown that whey solids can be used in candy, but to the candy manufacturer cost and convenience will be important factors entering any decision to use whey. Sweetened condensed whey probably is the most convenient economical and satisfactory form in which to process whey for the candy manufacturer.

**M26. Effect of the Cold Storage Temperature, Pasteurization Treatment, and Homogenization Pressure on the Properties of**

**Frozen Condensed Milk.** RAYMOND W. BELL, Bureau of Dairy Industry, U. S. Department of Agriculture.

Conditions which are optimum for the preservation of the flavor of frozen condensed milk of 8 per cent fat content may not be most favorable for the body of the product.

Best results were obtained when the condensed milk was (1) stored at minus 7° C., (2) heated to 76.5° C. and held 8 minutes, and (3) homogenized at 2,000 to 3,000 pounds' pressure per square inch.

**M27. Consumer Preference as Related to the Analysis of Vanilla Ice Cream in Tennessee.** THOS. B. HARRISON, H. B. HENDERSON, AND C. E. WYLIE, University of Tennessee.

Is there any relationship between the consumer preference for vanilla ice cream and the analysis of it? Does the consumer want the rich ice cream or is it more important for the flavor to be clean and the texture smooth? How much variation is to be found in Tennessee ice cream? A project is in progress at the University of Tennessee to determine the relationship of consumer preference to the analysis of ice cream.

Ice cream plant managers all over the state expressed a willingness to cooperate by sending one-gallon samples of vanilla ice cream and their laboratory record on fat, serum solids, sugar, gelatin, and eggs upon requests made by the secretary of the Tennessee Dairy Products Association.

Requests are made for the samples to be sent from six plants at one time. A pint sample is taken from each gallon for analysis, which includes the Mojonnier tests for fat and solids, the pH test by the Beckman meter, and the acid test. It was found that six samples are about all that the untrained, unprofessional judge of ice cream cares to pass judgment on at one time.

Stenographers, Extension staff members, Experiment Station workers, professors, and students are invited to express their preference on the six samples. They are asked to place the sample they like best first and the sample they like least at the bottom. Then they are to pick the second best and the next to the poorest. Then only two samples remain to decide upon. Such procedure makes their task less confusing. Each lot is judged by fifty or more people working independently of one another.

A table will be presented showing the results of this investigation as to formula, composition, and consumer preference. The forms used in recording the results and reporting to the cooperating companies will be shown.

**M28. The Use of Moving Pictures in Ice Cream Investigations.** W. H. REID, W. S. ARBUCKLE AND R. J. DREW, Missouri Agricultural Experiment Station.\*

Preliminary studies have been made in regard to the use of ordinary

\* Contribution from the Department of Dairy Husbandry, Missouri Agr. Exp. Sta., Journal Series No. 555.

colors and chromatic films taken with moving picture equipment showing the relation of variable mix compositions, effects of manufacturing procedure and subsequent treatment to which ice creams may be subjected.

This investigation has been limited to the application of moving pictures in studying the stability of ice creams. The ice creams and sherbets studied include those of common flavors, also fruit and nut ice creams.

The use of moving pictures permits acquisition of more complete detailed data than is obtainable when the usual procedure is followed. Availability of data so recorded emphasizes certain factors which may not otherwise be observed.

The report of this investigation will be illustrated by the use of moving pictures showing the effectiveness of this procedure.

**M29. Application of the Phosphatase Test to Determine the Efficiency of Pasteurization of Ice Cream Mix. A. J. HAHN AND P. H. TRACY, University of Illinois.**

Preliminary results of a study being made at this station have shown that the phosphatase test can be successfully used with certain limitations to determine the efficiency of pasteurization of ice cream mix.

Scharer's test<sup>1</sup> is being used for these determinations and a photoelectric procedure for determining color intensities.

Variations in temperature of pasteurization and in the holding period could be detected, while the use of raw ingredients as compared to pasteurized ingredients before pasteurization had no appreciable effect on the phosphatase content of the final pasteurized product.

Pure vanilla extracts added to the mix had no effect in the final results of the test, while imitation and artificial vanilla extracts increased the phenol value. The use of vanillin and coumarin crystals in ice cream mix, flavored to taste, produced striking results. Coumarin increased the phenol value over ten times that of the control sample, while vanillin produced a greenish tint which made it impossible to compare with the desired blue color.

Six different kinds of fruit flavored extracts were added to the ice cream mix and were found in five out of six cases to increase to a small degree the phenol value.

Three different kinds of cold pack fruits were added to the ice cream mix and in each case appreciably raised the phenol value of the test.

Storing mix during the aging period of two days at 40° F. decreased to a very small degree the phenol value of the test in the underpasteurized samples, but had no effect on the pasteurized samples. No change in the phenol value was noticed after the ice cream had been drawn from the freezer as compared to the aged mix. Storage in hardening room showed a slight decrease in phenol value for underpasteurized samples but no effect on pasteurized sample.

<sup>1</sup> JOURNAL OF DAIRY SCIENCE. Jan. 1938, p. 21.

**M30. Influence of Certain Mix Components Upon the Rate at which Freezing Occurs in Ice Cream as Measured by the Dilatometer Method.** W. C. COLE AND J. H. BOULWARE, University of California.

Dilatometer measurements were made on the rate at which freezing occurred in ice cream that was maintained during the observation at 2.5 degrees C. below the freezing point of the mix. For a given comparison it was necessary to adjust the freezing points of the mixes to the same value  $\pm 0.05^{\circ}$  C. This was done by adding sodium chloride in the proper amounts to those mixes whose original freezing points were above the lowest freezing point value in a given series of samples.

On the basis of this method the relative effectiveness of fat and milk-solids-not-fat in retarding the rate at which freezing occurred in ice cream was evaluated. It was found that the milk-solids-not-fat were more effective than fat in retarding the rate of freezing.

When these results were compared with the texture of ice cream made from the same mixes, the smoothness of the ice cream was improved to a greater extent by the milk-solids-not-fat than by the fat, provided ice crystal size was used as the basis of determining smoothness. Although deviations from this occurred when the organoleptic test was used as the basis of evaluating smoothness, the results from the two methods were generally in good agreement.

**M31. A Study of Quality Variations in Summer and Winter Made Cheese.** J. C. MARQUARDT, New York Agricultural Experiment Station.

Low quality in winter made cheddar cheeses causes numerous factories to operate at a loss during the winter period. The industry regards this as one of its main problems.

In New York, Wisconsin and the cheese sections of Canada the so-called winter cheese period extends from November 15th to May 21st. These dates may vary slightly with season. Data were collected in an organized manner regarding the quality of summer and winter made cheeses from the above mentioned sections. Experimental cheeses were also made to study these variations. The work thus far has revealed the desirability of expressing the degree of cheese flavor with the numerical score in comparing cheeses. The results obtained with the commercial and experimental cheeses indicate that the development of cheese flavor in summer made cheeses is much faster than in those made during the winter period. The numerical scores do not follow a comparable trend.

Cheeses made during both seasons were cured at temperatures derived from a cross index plan so that winter made cheeses were cured under summer conditions and vice versa. The cross index system supplied information on

curing temperatures beyond those normally encountered during the two seasons. These data are interesting and supply clues for curing procedures. They do not explain the variations obtained in winter and summer made cheeses. It is planned to cross index the temperatures at which milk is stored so that milk produced during both seasons will be subjected to comparable conditions. Increasing the calcium content of the milk increased the rate of breakdown in texture of cheeses made during both seasons.

**M32. Starters Used in Wisconsin Brick Cheese Factories.** WILLARD L. LANGHAUS, PAUL R. ELLIKER, University of Wisconsin.

In 1936 a survey of forty Brick cheese factories in Wisconsin revealed that a wide variety of starters were used to develop acid during the manufacturing process. A few operators carried starter cultures. Most of them, however, were using natural starters of various types. Troubles with starters and defective cheese were most commonly observed during the months of July and August. From the forty factories, observed in 1936, twelve were selected in August, 1937, for a more careful study, the results of which are reported here.

One day was spent at each of the twelve factories. Samples of starter, mixed milk delivered by each patron, and a composite sample of all milk delivered on that day were taken at each factory, iced and brought to the laboratory for analysis.

The approximate numbers and the dominant types of organisms in starter and milk were determined by means of a series of dilutions in litmus milk, followed by incubation at 20°, 37°, and 48° C., by means of Burri slopes and by microscopic examination. A week after the observations were made in each factory, a sample of cheese was obtained to determine the quality of the finished product.

Large numbers of thermotolerant types of acid-producing organisms were found in the starters. Yeast, gas-forming organisms of the *Escherichia-Aerobacter* group, as well as other undesirable types were commonly present, sometimes in large numbers.

There was a rather close correlation between the quality of the starter, as determined by the types of organisms present, and the quality of the cheese obtained from the factory. When starters of good quality were used in milk of inferior quality the chances of producing a superior types of cheese seemed to be greatly improved. The results of this study indicate that better cultures and improved methods of propagating starter are badly needed in Wisconsin Brick cheese factories.

**M33. Methods Which Help to Retain Fat in American Cheddar Cheese at High Temperatures.** HARRY L. WILSON, Bureau of Dairy Industry, U. S. D. A.

A method has been developed for manufacturing American Cheddar cheese so that the fat will not leak out when the cheese is held at relatively high temperatures. The method consists of separating the cream from the milk by adjusting the cream screw of the separator so as to produce cream having a fat content of 40 per cent, heating the resulting cream to from 100° to 142° F. and homogenizing it at from 500 to 2000 pounds' pressure per square inch. If the cream is not heated to 142° F. before it is homogenized, it is heated to 142° immediately after homogenization, held 30 minutes, and standardized to the fat content desired for making cheese with pasteurized skimmed milk. Or the homogenized cream can be standardized with raw skimmed milk and the mixture pasteurized immediately by heating to 142° and holding 30 minutes.

From this point on it is necessary to modify the manufacturing method slightly. The finished product will have all the characteristics of a normal American Cheddar cheese, except that there will be no oiling off nor leakage of fat when it is exposed to temperatures as high as 85° F.

#### M34. X-Ray Diffraction Analysis of White Specks in Cheddar Cheese.

S. L. TUCKEY, H. A. RUEHE, AND G. L. CLARK, University of Illinois.

Babcock, Russell, Vivian and Baer in 1902 described the conditions favoring the formation of white specks during the ripening of cheddar cheese. Val Slyke and Publow in 1910 claimed that the white specks were calcium soaps formed as a result of the combination of fatty acids liberated by bacteria acting only at low temperatures.

By x-rays analysis it has been shown that the white specks isolated from well ripened cheddar cheese have the same crystal structure spacings as calcium lactate. A few of the "d" spacings are listed:

"d" spacings in Angstrom units white specks	"d" spacings in Angstrom units standard calcium lactate
d <sub>1</sub> ..... 11.930	d <sub>1</sub> .. . . . 11.860
d <sub>2</sub> ..... 9.845	d <sub>2</sub> .. . . . 9.810
d <sub>3</sub> ..... 4.427	d <sub>3</sub> .. . . . 4.400
d <sub>4</sub> ..... 3.607	d <sub>4</sub> .. . . . 3.580
d <sub>5</sub> ..... 3.280	d <sub>5</sub> .. . . . 3.260
d <sub>6</sub> ..... 3.080	d <sub>6</sub> .. . . . 3.080

Since the "d" spacings are specific for the various substances, the white specks in cheddar cheese are apparently crystals or deposits of calcium lactate.

#### M35. Studies on the Ripening of Blue Cheese. C. B. LANE AND B. W. HAMMER, Iowa Agricultural Experiment Station.

Comparisons of cheese made from homogenized pasteurized milk and from homogenized raw milk have been continued. Pasteurization of the

milk should control certain defects sometimes present in cheese made from raw milk, especially during periods when the milk is of relatively poor quality. Cheese made from homogenized pasteurized milk did not show extensive fat hydrolysis of the odor of methyl-n-amyl ketone until after conspicuous growth of the mold, whereas cheese made from homogenized raw milk developed definite fatty acid and ketone flavors very early. Approximately 6 months of ripening were necessary before the cheese made from homogenized pasteurized milk developed a satisfactory blue cheese flavor; while only about 3 months were required with cheese made from homogenized raw milk.

The addition of a small amount of salt (2 per cent of the estimated weight of curd) to the curd, immediately before hooping, appeared to be a desirable procedure in the manufacture of blue cheese from homogenized milk. Cheese made from salted curd usually developed a more rapid and extensive mold growth than cheese made from unsalted curd, presumably due to the comparatively open texture, and the color was commonly less yellow.

Cheese made from milk produced by cows feeding on a ration high in cracked soybeans was regularly less colored than cheese made from ordinary milk. No definite difference in the flavor of the two types of cheese was apparent.

Loaf cheese, weighing about 5 pounds each, were made in special forms. The cheese ripened normally and the quality compared favorably with that of normal shaped cheese. The loaf cheese appears to be more practical than the usual cheese for retail stores where small slices are cut from a cheese.

**M36. Studies on the Vitamin A Content of Cheese. I. L. HATHAWAY AND H. P. DAVIS, University of Nebraska.**

The vitamin A content of twenty-two kinds of cheeses was studied by feeding these cheeses to rats whose body stores of vitamin A had been exhausted by being fed a vitamin A deficient diet. Twelve experiments were made in which approximately eleven hundred rats were used. From the data obtained it was evident that all cheeses do not have the same vitamin A value. Certain kinds appear to be good sources of this vitamin while other kinds are only fair to poor sources.

**M37. Plant Experience with Sonic Soft Curd Milk. LESLIE A. CHAMBERS, Eldridge Reeves Johnson Foundation and University of Pennsylvania.**

Homogenization of milk with the 360 cycle sonic oscillator has been studied under operating conditions in a few plants over periods of time ranging from three weeks to about one year. The data show that a unit handling milk at 250 gallons per hour with a power consumption of 4 kilo-

watts will reduce the fat particle size sufficiently to eradicate creaming. Simultaneously, there is a reduction of at least 50 per cent in curd tension with a final value never greater than 30 grams. Furthermore, there is a reduction of 50 per cent or more in the residual bacterial count after pasteurization and an improvement in both body and apparent flavor of the product.

Sonic homogenization has been compared directly with both high and low pressure methods. It has been found that both the sonic apparatus and low pressure homogenizers permit recovery of a much larger proportion of fat from returned milk than is the case when pressures above 2000 pounds are employed.

It should be emphasized that the terms "homogenized milk" and "soft curd milk" are not synonymous, since it is possible to obliterate creaming without having much effect on the curd tension. Many homogenized milks now marketed should not be called "soft curd."

The leucocytic sediment normally found in milks homogenized under conditions which produce the soft curd quality does not appear in bottles of sonized milk. This is possibly due to the fact that sonic cavitation completely disintegrates the white cells.

There is evidence that the phosphatase test is not valid when applied to homogenized milks since the processing apparently inactivates the enzyme wholly or in part.

**M38. The Digestibility of Natural and Processed Soft-Curd Milks. C. C. FLORA AND F. J. DOAN, Pennsylvania State College.**

The digestibility of natural hard-curd and soft-curd milk as well as that of soft-curd milk produced by boiling, by homogenization, by base exchange treatment, by enzymatic digestion, etc., has been determined, using an *in vitro* method developed by the authors but based on that of Doan and Welch. This method gives an indication of the rate of peptic breakdown (stomach emptying) and also of tryptic digestion which follows. The results obtained *in vitro* have been checked by feeding rats, kept for a period without food, and noting the progress of digestion by post-mortem examination of the digestive tract.

Briefly, the *in vitro* method consists in coagulating milk in small flasks, treating the coagulum with a digestion mixture of HCl and pepsin, agitating it mildly in an incubator at 37° C. and examining replicate flasks at definite intervals. The pH is adjusted periodically, beginning with approximately pH 6.0 and lowering to about pH 3.5 at the end of 2½ hours. Peptic digestion is measured by making nitrogen determinations on the material from the flasks which passes through a 12-mesh screen, at hourly intervals. At the start of the fourth hour the contents of the flasks are adjusted to about pH 7.0 with NaOH and trypsin is added. The pH is raised to about 8.1

at the end of the fourth hour and tryptic digestion is noted by making nitrogen determinations on tri-chlor-acetic acid filtrates at hourly intervals. In some cases sodium tungstate filtrates have been used in an effort to determine the degree of protein degradation.

Preliminary results indicate that natural soft-curd milk digests considerably faster than natural hard-curd milk in both peptic and tryptic digestion. Boiling milk retards the action of pepsin on the protein but the mechanism of physical breakdown in the stomach is more rapid and complete, so that by the method used, more rapid peptic digestion is indicated. Trypsin action is accelerated by the boiling of milk and total digestion at 6 hours is noticeably in advance of the unboiled milk.

The data covering other types of soft-curd milk are not sufficient to warrant conclusions at the time this abstract is being written but will be available for interpretation at the meetings.

**M39. The Relationship Between Curd Tension and Curd Size.** LESLIE A. CHAMBERS AND IRVING J. WOLMAN, Eldridge Reeves Johnson Foundation and University of Pennsylvania.

Apparatus has been developed for studying *in vitro* curd formation under controlled conditions approximating those in normal and pathological human stomachs. With the new technique it is possible to measure the total curd surface presented for gastric digestion and thus to evaluate measurements of curd tension as indices of digestibility.

When curd surface areas are compared with the usual curd tensions there is general agreement with the theory that curd tension is an index of the rate of gastric clearance, but there are exceptions to this rule in the cases of milks subjected to drastic additions or subtractions. Thus, diluted milk while lowered in curd tension, gives about the same surface number as the undiluted base.

We have examined several hundred samples including most of the available types of certified, raw, pasteurized, homogenized, sonized, enzyme treated, base exchange processed, condensed, evaporated, and dried milks as well as a wide variety of modifications used in the feeding of infants. The data permit a preliminary evaluation of the curd-forming properties, free from any limitations of the curd tension test.

**M40. Artificial Gastric Digestion of Milk.** MAURICE E. HULL, M. & R. Dietetic Laboratories, Inc., Columbus, Ohio.

Since the development and use of the Hill test for determining curd tension of milk there has been an extensive use of this method by the dairy industry. It has caused an advance in the knowledge of how milks behave in the animal stomach. Milks of low curd tension have been shown to be beneficial in human digestion. However, it has been difficult to measure

digestion. In order to shed some light on gastric digestion of milks the author has developed an artificial method of studying this function of the stomach.

The method was designed for the formation of the curd at the optimum reaction for the production of an enzyme curd followed by changing this reaction to one for optimum pepsin digestion. Gentle agitation was maintained throughout the method. The degree of digestion being measured by a protein determination of the filtered digestion mixture.

### GENERAL SESSION, WOOSTER

#### 3. **The Inter-Relationship of Production and Manufacturing Research in the Development of the Dairy Industry.** ERNEST L. ANTHONY, Dean of Agriculture, Michigan State College.

The dairy industry is the wonder child of American Agriculture. Less than a century ago it was unknown, unhonored, and rather despised as an occupation or activity,—principally limited to women, the kitchen, and the backyard. From this humble and insignificant start, it has steadily and with increasing momentum come from behind to be the present day leader of the several important phases of American Agriculture.

This phenomenal advancement or change has been largely the result of the consistent application of the sciences to its problems. It is no idle boast to say that no other phase of agriculture has been so completely influenced and guided by scientific principles as has the dairy industry. These principles have been adopted and applied through the medium of research. This research has not been the development in the production field or the manufacturing field alone but fortunately has been advanced in both fields through close coordination. This close coordination or inter-relationship has made possible a united front on the problems of the industry. The research work on vitamin introduction through production methods and its retention through processing and distributing procedure; the studies in quality incorporation in production and its value in manufacturing practices; the researches in consumer demand and market limitations and the adjustment in production methods to satisfy the problem are only a few typical examples of the close inter-relationship which has been so important in the dairy development of the last half a century.

### PAPERS READ BY TITLE

#### 1. **The Relation of Milking Machines to the Incidence of Mastitis.** EDWARD B. MEIGS, HENRY T. CONVERSE, Division of Nutrition and Physiology, and LLOYD A. BURKEY, MORRISON ROGOSA, AND GEORGE P. SANDERS, Bureau of Dairy Industry, U. S. Department of Agriculture.

The introduction of machine milking in a herd of cows maintained for nutrition work by the U. S. Department of Agriculture was followed by a severe outbreak of mastitis. During the outbreak and after it had subsided, the chlorides and the numbers of leucocytes and bacteria were determined in numerous samples of milk while the cows were on various modifications of machine milking and on hand milking.

The modifications of machine milking chiefly studied were "severe machine milking," in which the vacuum used was high, the machines were left working on the udders for rather long periods, and the cows were stripped by massaging the udders while the machines were still working on them; and "mild machine milking," in which the vacuum and periods of milking were lessened and the cows were stripped by hand. Some of the cows milked by all methods had had previous cases of mastitis, and some had not.

In the milk of the cows milked by hand, the chlorides and the numbers of leucocytes and bacteria have remained at low levels and the milk yields have been normal in almost all instances, whether or not the cows had had previous cases of mastitis. For the cows subjected to severe machine milking, the results have been variable; but, in almost all instances, high leucocyte counts have appeared in the course of a few days or a few weeks, which usually were not accompanied immediately by increased bacterial counts. After a further variable interval of several days or weeks, large numbers of bacteria have appeared in the milk in the majority of instances, accompanied by a great increase in chloride content, rapid reduction in milk yield, and other signs of acute mastitis. A change to hand milking was uniformly followed by a rapid reduction in leucocytes and chlorides, and, in the next lactation period, by a marked recovery in milk yield and by a return of the milk to normal in practically all respects. Mild machine milking has not resulted so far in any severe cases of mastitis, though the leucocytes, and sometimes the chlorides, have tended to be definitely higher than in the case of hand milking.

The tendency of machine milking to produce high leucocyte counts and increased chloride content without accompanying high bacterial counts, and the tendency of hand milking to produce opposite effects, constitute strong evidence for the view that machine milking may sometimes have a mechanically injurious effect on the secretory membranes of the udder. The frequency with which these symptoms of injury have been followed by acute mastitis in the case of severe machine milking indicates that the injury, if carried far enough, renders the udder tissues a better medium for bacterial growth.

It should be emphasized that only one type of milking machine has been studied so far, and that no severe cases of mastitis have followed its use, except under the conditions of "severe machine milking" or conditions approaching thereto. The results should by no means be taken to prove that machine milking in general will be accompanied by a greater incidence of mastitis.

**2. Sudan Grass Hay vs. Clover Hay for Dairy Cows.** C. E. WYLIE AND S. A. HINTON, University of Tennessee.

Years of drought frequently require the use of emergency crops in feeding dairy herds. Sudan grass is such a crop since it may be grown late in the summer. It makes a rapid growth and great tonnage. It is of value, therefore, to obtain information on its feeding value even for a short time.

During the winter of 1936-37 an experiment to determine the value of Sudan grass hay for dairy cows was set up at the University of Tennessee. Two groups of Jersey cows, four cows in each group, were used. Group I was fed a ration of grain, silage, and Sudan grass hay. Each cow received 20 pounds of silage daily. Grain was fed each cow at the rate of one pound of concentrates to three pounds of milk produced. Each group was fed all the hay that they would eat. Accurate weights of the amounts of feed fed and refused was kept and recorded daily. At the end of forty-five days the kind of hay that each group was fed was reversed, Group I receiving Sudan grass hay and Group II receiving clover hay. Milk weights were kept and the milk was tested one day each week for butterfat. The cows were weighed one day each week and weights recorded. On the basis of this short time experiment, Sudan grass hay was worth approximately five-sixths as much as clover hay as a feed for dairy cows. The cows in both groups maintained normal body weight during the experiment. The cows were never off feed and kept in good physical condition. All of the cows bred and calved normally.

**3. The Extraction and Assay of the Hormones of Cattle and Sheep Pituitaries.** A. J. BERGMAN AND C. W. TURNER, University of Missouri.\*

It has been shown that the milk yield of dairy cattle can be increased by the injection of certain anterior pituitary extracts. Since it is assumed that the hormones of the pituitary directly (lactogenic) and indirectly (thyrotropic, parathyrotropic, adrenotropic, carbohydrate, fat and protein metabolism) influence lactation, considerable attention has been given to the chemical extraction, separation, purification and biological assay of these factors. In order to investigate the relation of the several hormones to lactation, it is desirable to have available assayed pituitary extracts.

All of the hormones mentioned have not been separated nor have assay methods been developed. However, assay techniques for the lactogenic, thyrotropic, carbohydrate metabolism and gonadotropic hormones have been developed and the units defined. For assay of the lactogenic hormone the common pigeon is used; for the thyrotropic and carbohydrate metabolism the immature male guinea pig. Since the gonadotropic hormone is considered as

\* Contribution from the Department of Dairy Husbandry, Missouri Agr. Exp. Sta., Journal Series No. 561. Aided in part by a grant from the Committee on Research in Endocrinology of the National Research Council.

a contaminant as far as lactation is concerned, its presence must be determined. For this test animal the day old chick is used.

The anterior lobe of both sheep and cattle pituitaries have been used as a source of the hormones. The anterior lobes are ground, dried with acetone and assayed for the lactogenic, thyrotropic, carbohydrate metabolism, and gonadotropic content. Approximately 85-90 per cent of the inert material is then removed by extraction with alcohol at pH 9-10. This extract referred to as the *initial*, is again carefully assayed. The lactogenic hormone (I) is then separated from the thyrotropic, carbohydrate metabolism and gonatropic hormones (II). It (I) is then further purified and upon assay has been shown to be relatively free of other hormones. The fraction containing the thyrotropic, carbohydrate metabolism, and gonadotropic (II) is then assayed. Further attempts are being made to separate this fraction. The lactogenic (I) potency has been increased from 500 to 6600 units per gram. The fraction containing the thyrotropic, carbohydrate metabolism and gonadotropic (II) has been increased as follows: thyrotropic, 50 to 4000 units per gram; carbohydrate metabolism 20 to 660 units per gram; and the gonadotropic from 250 to 10,000 units per gram.

#### 4. Relation of Lactic Acid and Glucose of the Blood and Glycogen in the Mammary Gland to Milk Secretion. W. E. PETERSEN, J. C. SHAW, University of Minnesota.

It has been shown recently that the active mammary gland removes both lactic acid and glucose from the blood. From this it was postulated that glucose and lactic acid are used by the mammary gland for the synthesis of lactose. This hypothesis was proven to be correct by Peterson and Shaw who succeeded in synthesizing lactose *in vitro* from glucose plus lactic acid and mammary gland tissue. Lack of a definite relationship between the amounts of glucose and lactic acid removed by the mammary gland from time to time suggest some other mechanism is involved in the process of lactose synthesis.

These variations could be accounted for by a storage of carbohydrate in the gland. Analysis of both lactating and non-lactating glands showed an average of 0.2 per cent of glycogen on the basis of the total weight of the gland. The glycogen, temporarily stored in the gland, is therefore probably built up from the blood carbohydrate when large amounts are taken out by the gland and becomes a source of the lactose precursors when small amounts of carbohydrate are taken out of the blood.

#### 5. The Carotene Requirement of Dairy Calves. RUEL E. WARD, S. I. BECHDEL, AND N. B. GUERRANT, Departments of Dairy Husbandry and Agricultural and Biological Chemistry, Pennsylvania State College.

In an effort to determine the carotene requirement of growing calves, twenty-seven animals (20 Holsteins and 7 Guernseys) have been fed varying amounts of carotene from the following sources: Alfalfa hay, timothy hay, corn silage, alfalfa silage, yellow corn, and a commercial carotene preparation (Puratene). The carotene content of the different materials was determined by the Struve modification of the Guilbert method.\* When alfalfa hay or Puratene (carotene in cottonseed oil) was used as the source of carotene, 12 to 14 micrograms of carotene per pound body weight per day was sufficient to prevent the symptoms of vitamin A deficiency. These results agree very well with those reported by Hart and Guilbert. When timothy hay or corn silage was given as the source of carotene the requirements were about 25% higher than the values mentioned above. The results to date with alfalfa molasses silage indicate that the requirement may be even greater than this latter value. The differences noted above are probably due to the fact that with some materials the method of carotene estimation is complicated by the presence of carotenoids other than beta carotene.

Increased growth or improved well being in Holstein calves did not result when the carotene was supplied above 20 to 30 micrograms per day per pound body weight. The results to date indicate that minimum requirements of carotene for Guernsey calves are not appreciably higher than for Holstein calves. The Guernseys, however, appear to be more subject to intestinal and respiratory disorders at low carotene intakes. The general results of the experiment indicate that calves have a slightly higher requirement for carotene in winter as compared with the summer months. Work with older animals indicates that heifers, one to two years of age, will probably show little or no carotene deficiency in the winter months if they have been on good pasture during the previous summer.

#### 6. Revised United States Standards for Quality of Creamery Butter.

Roy C. Potts, Principal Marketing Specialist, United States Bureau of Agricultural Economics.

The Revised Tentative United States Standards for Quality of Creamery Butter became effective April 1, 1938 by approval of Dr. A. G. Black, Chief of the Bureau of Agricultural Economics.

By the old standards the five factors, flavor, body, color, salt, and package, were each given an arithmetical rating and the total of those ratings became the final score. Under the Revised Standards the factor of package is eliminated from consideration entirely. The factors of flavor, body, color, and salt are each rated entirely independent of the other, and the final score is determined by the application of a rule and a schedule for defects in body, color, and/or salt which are permitted in butter of a particular flavor rating and do not require the final score to be below the rating given to flavor.

\* Permission obtained from Oscar H. Struve, Chemist, Eastern States Feed Mills, Buffalo, New York, to use the method in this study.

The official United States score of individual sample of creamery butter under the Revised Standards is determined by the following general rule: "The official United States score of an individual sample of creamery butter shall be determined by deducting from the flavor rating of the sample the amount that the total ratings of the defects in body, color, and salt is in excess of the ratings for defects permitted in these factors for butter of the particular flavor rating, the official United States score to be expressed as a whole number by lowering any half score to the next lower full score."

To properly apply the Revised Standards, a grader must know the rating established for each identified flavor and he must be able to identify each flavor correctly with respect to its character and its degree or extent of development. Without such knowledge of the various identified flavors a grader would not be able properly to apply the standards for he would not know them. The grading of butter according to these standards, therefore, is not a job for a layman, who has a general knowledge of butter and a generally good taste sense, but if it is to be expertly and properly done, it requires the expert knowledge and the expert experience of a completely and competently trained personnel.

**7. Effect of Temperature and Composition Upon the Physical Properties and Dipping Qualities of Ice Cream.** W. H. E. REID, R. J. DREW AND W. S. ARBUCKLE, University of Missouri.\*

This study treats with the composition of the ice cream mix and the effect of serving temperature of vanilla ice cream upon the crystalline structure, stability, consumer acceptance, dipping and keeping qualities of ice creams.

Ice creams varying in fat content from 12 to 16 per cent; serum solids content from 9 to 15.5 per cent; sugar content from 12 to 18 per cent and gelatin content from .20 to .50 per cent were observed at the serving temperatures of 6, 10, 14 and 18 degrees Fahrenheit.

It was observed that as the serum solids of the mixes were increased there was a relative increase in the pH. Variation in the composition of the mixes had no marked effect upon the surface tension. Increasing the solids of the mixes resulted in a relative increase in the viscosity.

The serving of the ice creams at a temperature of 10 degrees Fahrenheit was considered desirable from the consumer's viewpoint. Ice creams containing 14 per cent fat, 13 per cent serum solids and 14 per cent sugar were preferred of the series of mixes studied.

Microscopic examination of the ice creams indicated that as the percentage of fat, serum solids, sugar or gelatin was increased the ice crystals appeared relatively smaller. Macroscopic pictures revealed that increased fat and gelatin percentages increases the stability, while the effect of varia-

\* Contribution from the Department of Dairy Husbandry, Missouri Agr. Exp. Sta., Journal Series No. 557.

tion in serum solids and sugar content on stability was influenced greatly by the serving temperature.

Dipping studies show that the serving temperature and composition of the mix affect the number of scoops secured per gallon. The greater number of scoops of ice cream was obtained at a temperature of 6 degrees Fahrenheit. The least number of scoops was usually obtained as the serving temperature was increased, there being a slight decrease in the number of servings per gallon.

The keeping qualities of all ice creams was most favorable when held at a temperature of 6 degrees Fahrenheit. Sandiness did not become apparent in the ice creams containing 13.50 per cent serum solids even when held at the different serving temperatures for three weeks. However, the mixes containing 15.00 per cent serum solids showed indications of sandiness when aged two weeks.

Ice creams served at 10 degrees Fahrenheit had the most desirable eating qualities although a temperature of 14 degrees Fahrenheit was considered desirable. Ice creams served at 6 degrees were described as being too cold with the flavor submerged.

#### 8. A Comparative Study of Metal and Glass Petri Dish Covers. HERBERT JENKINS, New England Dairies, Inc.

In commercial laboratories where large numbers of standard plate counts are made daily, the breakage of petri dishes is an expense item to be considered. In order to eliminate a portion of this breakage, a search was made for a substitute for the glass cover. Obviously there could be no substitute for the glass bottom.

Experimental work was undertaken, first, to find a material for the cover which would be non-breakable; second, to determine whether this material would give the same results bacteriologically as glass.

A metal aluminum cover was finally adopted. Metals other than aluminum proved unsatisfactory because of appearance, rusting, warping, weight, discoloration, or the fact that they did not take identification marks easily. Covers of special paper were eliminated because of their expense and the fact that they allowed the agar to dry. Standard plate counts were made in duplicate, using glass and aluminum covers on more than 2,000 samples of milk of varying quality. No difference in the counts, other than the usual variations encountered with the standard plate method, was observed whether glass or aluminum covers were used.

Since results are the same with aluminum, as with glass covers, aluminum petri dish covers have several distinct advantages:

1. They are non-breakable and practically indestructible. For this reason they will last indefinitely, making the unit cost very low.
2. Their original cost is approximately one-third the amount of the pyrex cover.

**9. Summary of Experiment with the Delaval Standardizer.** J. H. FRANDSEN, Massachusetts State College.

1. Standardization reduces the amount of visible sediment in milk.
2. In general, standardization increases fat and total solids and decreases specific gravity.
3. Standardization has little effect upon bacteria count.
4. Standardized milk tends to have a better flavor than milk of the same age not standardized.
5. Standardization with a mechanical standardizer is more practical and economical than standardization by siphoning or foremilk.

**10. Methylene Blue Reduction Time as an Indication of the Suitability of Milk for the Manufacture of Swiss Cheese.** A. B. EREKSON, C. A. ECKBURG AND E. LEE, The Borden Company.

From two to eight wheels of Swiss cheese were manufactured daily with a few short interruptions in one factory in Clark County, Wisconsin, during the period April 1, 1935 and March 31, 1937. A total of 2463 cheeses were included in the study. Each kettle of milk was sampled before the starter was added and methylene blue reduction tests were made under carefully-controlled conditions. A complete set of records was kept on the manufacturing procedure and the quality of the cheese. The tabulated results indicate a very definite relationship of methylene blue reduction time of the milk to the quality of the cheese with a decided tendency toward better cheese as the reduction time increased up to six hours. The results show that when the reduction time was less than three hours, only one cheese in every 10.08 manufactured was above a "C" in grade, while when the reduction time was over three hours, one cheese in every 3.88 made was either a "B" or an "A" grade.

**11. Casein Milk Fat as a Foam Depressant in Casein-Clay Slips.** G. A. RICHARDSON AND N. P. TARASSUK, College of Agriculture, Davis, California.

The authors have previously concluded that the relationship between the fat content of casein and its foaming tendencies was somewhat obscure.<sup>1</sup> They later reported that oxidized fat is much more effective in preventing foaming of casein-clay slips than fresh fat.<sup>2</sup> It was postulated that this might account for the decrease in foaming tendency of casein on aging.

Additional studies confirmed this theory and showed that the physical and chemical condition of the fat plays the major, if not the only, rôle in the non-foaming tendency of casein-clay slips.

The spreadability of fresh milk fat on water, sodium casein solutions, and casein-clay slips shows a much lower value than does milk fat which has

<sup>1</sup>JOUR. DAIRY SCIENCE, 20, 449, 1937.

<sup>2</sup>Proc. 23rd Annual Meet, Western Div. Amer. Dairy Sc. Assoc., pp. 70-78, 1937.

undergone chemical deterioration. This fact explains the foam-depressing action of the fat of commercial casein.

**12. The Relationship of Mastitis Milk and Soft-Curd Milk to the Manufacture of Swiss Cheese.** KENNETH J. MATHESON, LLOYD A. BURKEY, GEORGE P. SANDERS, AND ROBERT R. FARRAR, Bureau of Dairy Industry, U. S. Department of Agriculture.

In order to study the effects of mastitis milk and soft-curd milk on the manufacture of Swiss cheese a detailed survey was made of the milk from individual cows. As a result of this survey the milk was divided into four general groups, as follows: (1) Normal milk; (2) Mastitis milk; (3) Soft-curd milk with slow rennet coagulation, not mastitis; and (4) Normal low and high rennet-curd-tension milks.

As a result of manufacturing cheese from these types of milk, the following conclusions are drawn:

That mastitis milk causes the cheese to overset more than normal milk.

That there is evidence that the mastitis milk has an inhibitory effect upon the development of the lactobacillus (39a).

That there are more glass defects in the normal-milk cheese than in the mastitis-milk cheese.

That cheese made from normal milk has a lower percentage of water than that made from mastitis milk, or with the mixture of mastitis and soft-curd milks.

That the coagulation period is lengthened by the use of mastitis milk as compared to normal milk.

That grades of cheese from mastitis milk, from the mixture of mastitis milk and soft-curd milk, and from the soft-curd milk alone, are better than those of cheese from normal milk.

Of the cheeses made from milk produced by cows free of mastitis, the low rennet-curd tension milk cheese was higher in moisture, softer in texture, and poorer in quality than that made from high rennet-curd-tension milk.

It is to be understood that this question has been attacked solely as a research problem in manufacturing, and no implication is to be made regarding its possible public health significance.

**13. Texas One Day Dairy Shows.** G. G. GIBSON AND E. R. EUDALY, Texas A. & M. College.

The one day Dairy Show or "Dairy Day" was the result of a demand on the part of dairymen, county agents and representatives of milk plants for a program that would recognize excellence in dairy type, production and products.

In planning the Dairy Shows or "Dairy Days" the idea of having a program that would interest both the dairyman and his family was kept in mind.

The main feature of the day was the cattle show. Cattle were classified into Class A, B and C. Ribbons were awarded for each of the classes. At each show the best female and best bull were selected. Certificates were awarded where animals met certain standards of type and production. .

A Dairy Cattle Judging Contest for 4-H Club members, F.F.A. members, men and women was carried along with the classification of the cattle. A Dairy Products Contest for women was also included. Samples of milk, cream and butter were scored. Between classes short talks were given by men from Texas A. & M. College and others dealing with problems that concerned the dairymen in that area.

In planning the dairy days a series of preliminary meetings had been held at which committees were appointed to handle the arrangements for each Show. It was left up to the various committees to work out a program for their Show that would best serve their interests.

#### 14. The Texas Trench Silo Program. G. G. GIBSON, Texas A. & M. College.

While it has been known for many years that feed can be stored satisfactorily in a trench silo, no concerted effort was made in Texas to bring about a wider use of the trench silo until 1932. In 1932, 523 trench silos were reported by county agents. In 1937 there were 9,483 trench silos containing almost 1,000,000 tons of silage reported in Texas.

In order to arouse interest in and to sell the idea to farmers, several methods have been employed:

(1) Trench silo demonstrations have been set up in various parts of the county. The County Agent arranges a meeting at the trench at time of filling. Other meetings are arranged at the trench when the silage is being fed so that farmers will have an opportunity to see the silage being used.

(3) Trench silo posters have been prepared. .

(4) Chambers of Commerce and other organizations have sponsored contests within counties and within certain sections of the state based on increasing the number of trench silos. Other counties have increased the number of trench silos through the effort of the local committees.

(5) Commissioners' Courts in a number of counties have rendered assistance by making equipment available for the construction of trench silos. In the ranch country, equipment used for building tanks in connection with the range program is available for trench silo construction.

(6) The fact that trench silos are adaptable to any kind of conditions and practical for any farm has been kept uppermost at all times. It has been demonstrated that all kinds of crops in addition to regularly recognized silage crops can be stored in a trench silo. Many trenches have been filled in Texas with whole bundle feed so that it is possible for a farmer to have silage who does not have any equipment of any kind for cutting or handling the feed.

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## ABSTRACTS OF LITERATURE

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## ABSTRACTS OF LITERATURE

### BACTERIOLOGY

- 225. Bactericidal Property of Milk.** RALPH B. LITTLE, Rockefeller Institute, Princeton, N. J. 25th Ann. Report of Intern. Assoc. Milk Sanitarians, p. 105, 1936.

All milks studied had a substance present which inhibited the growth of non-hemolytic mastitis streptococci and known cultural strains of scarlet fever streptococci. This inhibitory effect was noticeable for several hours. The concentration of the inhibitory principle varies in the secretions of different animals and frequently in the quarters of the same animal.

It is assumed that the substance is produced in the udder of the animal and that its function is primarily a natural means of restraining the growth of bacteria in the udder.

Whether or not this substance is potent for many kinds of bacteria is not known. However, we must realize the opportunity available for the entrance of bacteria into the teat canal, yet, in the main, the flora of the udder is limited to relatively few species; and these species have probably become well adapted to the environment and to the influence of this inhibitory substance. L.H.B.

### BUTTER

- 226. Regulatory Problems Relating to the Manufacture of Butter.** CHARLES S. TRIMBLE, U. S. Bureau of Dairy Industry, Washington, D. C. 25th Ann. Report of the Intern. Assoc. Milk Sanitarians, p. 311, 1936.

A discussion of some of the phases of butter manufacturing which require supervision by regulatory agencies. L.H.B.

- 227. The Cold Storage of Butter.** F. BROCHOT, *Le Lait* 18, 171, p. 23, Jan., 1938.

Refrigeration equipment in cold storage chambers for butter is discussed. The importance of maintaining a low humidity in the butter storage chamber is emphasized. In putting butter into storage for long periods, the work indicates that the butter be prepared from cream pasteurized at a high temperature and manufactured carefully and not permitting contact with copper. The butter storage chamber should be free from mold and should be washed with chlorine solution before storage of the butter. Light should be excluded from the butter storage chamber. Condensed water from cold surfaces in the cold storage chamber should be eliminated as far as possible.

A.H.J.

228. **The Diacetyl in the Butters of Normandy.** CH. BRIOUX AND EDG. JOUIS. Agronomic Sta. of the Lower Seine. *Le Lait* 18, 171, p. 11, Jan. 1938.

The diacetyl and acetyl methyl carbinol contents of a considerable number of butters were determined by the method of Pien. Determination of acetyl methyl carbinol was accomplished by introducing 100 grams of the butter into a 300 to 400 cc. flask, adding 20 cc. of iron perchloride and distilling slowly with steam until 50 cc. of distillate were obtained. Ten cc. of the distillate were then used in the colorimetric determination of the contained diacetyl according to the method of Pien. Fresh Norman butters contained normally a small quantity of diacetyl, usually from 0.05 to 0.5 milligrams of diacetyl per kilogram and in rare cases as much as 2.5 milligrams of diacetyl per kilogram of butter were found. Butters made in cooperative or industrial creameries contained considerably more diacetyl than butter made on the farm. The diacetyl normally contained in fresh butter disappeared rapidly on storage of the butter. Fifteen to 18 days after manufacture of the butter, the diacetyl content decreased to  $\frac{1}{10}$  its original value. The acetyl methyl carbinol contents of butter are considerably higher than the diacetyl contents, usually between 10 and 30 milligrams per kilogram of butter, but some butters may contain as high as 69 milligrams per kilogram. The acetyl methyl carbinol did not appear able to give rise to an appreciable quantity of diacetyl. The diacetyl and acetyl methyl carbinol contents of fermented cream containing 95.2 milligrams of acetyl methyl carbinol per kilogram yielded butter and buttermilk containing 42.4 and 195.3 milligrams of acetyl methyl carbinol per kilogram respectively. The same cream containing 1.92 milligrams of diacetyl per kilogram yielded butter and buttermilk containing 1.50 and 3.10 milligrams of diacetyl per kilogram respectively.

A.H.J.

Other abstracts of interest are numbers 246, 251, and 254.

## CHEESE

229. **Cheese Vats of Non-oxidizable (Stainless) Steel.** JOSEF KRENN, School and Federal Exp. Sta. for the Dairy Industry, Wolfpassing, Austria. *Le Lait* 18, 171, p. 1, Jan., 1938.

It was noted that when curd was prepared in non-oxidizable steel vats, the curd adhered to the walls of the vat and formed a spongy coagulum while when copper vats were used the curd drew away from the wall and formed a more desirable hard curd free from whey. It was not that this phenomenon was due to the probability that in the case of copper containers, minute traces of the copper walls dissolved allowing thereby the curd to draw from the walls. This explanation was found to be incorrect. It made no difference whether raw milk, pasteurized milk or skimmilk used in the vats, the curd in all cases adhered to the stainless steel or nickel chromium vat, while it drew away from the copper vat. Modifying the acidity of the milk, or the temperature or the

amount of rennet had no effect. The curd adhered to glass containers but not to glazed porcelain. The state or condition of the surface of the inoxidizable steel (degree of polishing) was without affect on the adherence of the curd to the surface. It appears to be a particular property of the inoxidizable steel surface that curd presents a particularly high attraction for it. In order to suppress this attraction, it is sufficient to grease carefully the inoxidizable steel vat with butyrin. The cheese curd then detaches itself as easily and as freely from this greased surface as from a copper vat. A.H.J.

## CHEMISTRY

230. **Some Observations on Chlorine and Metals.** FRED M. GRANT, Bureau of Dairy Industry, U. S. Dept. of Agr., Washington, D. C. 25th Ann. Report of Intern. Assoc. Milk Sanitarians, p. 9, Oct., 1936.

This paper presents the observations made on the corrosive action of a chloramin-T compound and of a calcium hypochlorite and sodium carbonate mixture on seven different metals, tin, black steel, Monel metal, allegheny metal, copper, aluminum and bronze. The solutions used were of a strength of 200 ppm. of available chlorine. Weighed metal strips were used all of such size that an equal surface was presented in each instance.

It was found that bronze and copper were affected most by the chloramin-T and least by the hypochlorite solution. Tin, Monel metal and allegheny metal were practically untouched by the chloramin-T, but Monel metal and tin were mildly effected by the hypochlorite solution. Aluminum, although strongly attacked by hypochlorite, was only mildly effected by chloramin-T. L.H.B.

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

231. **The Progress of the American Industry with Compounds of Lactic Acid.** G. GENIN, Paris, France. *Le Lait* 18, 171, p. 43, Jan., 1938.

Milk is freed of its fat and much of its protein. The remaining serum is the source of the sugar for fermentation. The appropriate organisms are added to this serum, and in 10 to 24 hours the lactose is converted into lactic acid. In the course of the fermentation, lime is added from time to time. During the fermentation the temperature is held at about 100° F. When the fermentation is complete, the liquid is neutralized with lime and heated to 180° F. to 220° F. On cooling a slime separates out. The liquid containing the calcium lactate is siphoned off and concentrated under vacuo. After cooling calcium lactate separates from the condensed liquid. Purification is effected by washing the crystals in a centrifuge. The crystals are then dissolved and the solution heated to 140–160° F., the solution also having its pH raised to 10–12 by the addition of calcium oxide. After standing 2–3 hours, the last impurities separate as a slime. The supernatant liquid is

drawn off and neutralized with lactic acid. A further purification may be made by the use of decolorizing carbon. The purified solution is finally cooled and the calcium lactate crystals obtained by centrifugation. A modification of this process is also described. Sodium lactate may also be prepared by treating a solution of calcium lactate with sodium sulphate. The precipitate of calcium sulphate is filtered off and the sodium lactate concentrated under vacuo to a syrupy consistency. Lactic acid can also be prepared from calcium lactate by adding sulphuric acid to a solution of calcium lactate. The lactic acid thus obtained has a slight brown color. This color can be removed by treatment with decolorizing carbons or by treatment with potassium ferrocyanide.

A.H.J.

- 232. Concerning the Use of Casein in the Fabrication of Plastic Materials.** G. GENIN, Paris, France. *Le Lait* 18, 171, p. 45, Jan., 1938.

A review of the development of plastic materials from casein is given with special reference to the often overlooked work of Trillat.

A.H.J.

Other abstracts of interest are numbers 251, 252, 253, 256, and 257.

## DISEASES

- 233. The Present Status of Bang's Disease in Man.** RICHARD KERN, Univ. of Penn., Philadelphia, Pa. 25th Ann. Report of Intern. Assoc. of Milk Sanitarians, p. 248, 1936.

This paper gives a very complete history of Bang's disease.

Up to 1935, 9,965 cases have been reported. However, it is apparent that not all cases are reported.

As a result of some of the work done by the government during the last few years, it was found that of 3,317,760 cows examined in this country, 381,657 were reactors, an incidence of 11.2 per cent. Nearly 50 per cent of all herds examined have some reactors.

The disease may be contracted by ingestion of infected raw milk or by contact. Pasteurization will take care of the milk infection, but the underlying problem is eradication of the disease in animals; this must be accomplished.

L.H.B.

- 234. Milk-Borne Streptococci Infections.** E. L. STEBBINS, H. S. INGRAM AND E. A. REED, Div. of Communicable Diseases, State Dept. of Health, Albany, N. Y. *Am. J. Pub. Health* 27, 12, p. 1259, 1937.

An analysis was made of 1,529 cases of milk-borne streptococcus infections occurring in 7 epidemics in New York State during 1934-1936. In 6 epidemics, the source of contamination of the milk supply was a cow suffering from an acute mastitis caused by a hemolytic streptococcus of the type usually

associated with human infection (Lancefield Group A) and there was suggestive evidence of a human source of the bovine infection in each instance. The clinical and epidemiological observations are discussed. M.W.Y.

**235. Undulant Fever in Milk, and Its Relation to Bang's Disease in Livestock.** G. W. ANDERSON, Mass. Dept. of Public Health. Boston, Mass. *J. of Milk Tech.* 1, 1, p. 26, Oct. 1937.

The existence of infection through the medium of raw milk must be recognized. The responsibility of the health officer is discussed.

L.H.B.

**236. An Outbreak of Septic Sore Throat in Bergen County, (N. J.).** W. H. MACDONALD, N. Y. State Dept. of Health. 25th Ann. Report of Intern. Assoc. of Milk Sanitarians, p. 180, 1936.

In a period of 27 years since 1909 the New Jersey State Dept. of Health investigated 58 disease outbreaks traceable to milk. Fifty-seven of them were traced to raw milk, the other one was traced to pasteurized milk wherein the bottles were hand-capped (20 years ago before machine capping was required) by a person with a mild case of typhoid fever.

Not until 1934 was septic sore throat listed among the milkborne epidemics. Since then three such epidemics have occurred with 325 or more cases and 9 deaths. The last outbreak occurred in April and May, 1936 when 175 cases or more had occurred and there were seven deaths.

L.H.B.

**237. The Present Status of Milkborne Diseases. Report of Committee on Communicable Diseases Affecting Man.** J. G. HARDENBERG. 25th Ann. Report of Intern. Assoc. Milk Sanitarians, p. 120, 1936.

There has been no definite trend in recent years in milkborne epidemics (since 1923).

However, in the past five years the number of deaths reported have averaged less than one per epidemic, whereas prior to that the ratio of deaths to epidemic was 1.3 to 1.

In 1935 there was a total of 43 milkborne epidemics reported in the United States and 2 in Canada. With a total of 1846 cases and 21 deaths.

Typhoid fever and septic sore throat were responsible for 58 per cent of the epidemics, 64 per cent of the cases and all of the deaths.

The majority of the epidemics in the U. S. occurred in small communities; 27 were in towns of less than 5,000 population, 10 in towns of 5,000 to 25,000, four in cities of 25,000 to 50,000 and only two in cities of more than 100,000.

Raw milk as usual was the chief offender.

Pasteurized milk was involved in five cases but in three of them some raw milk was also consumed and in two of them the supplies were improperly pasteurized.

In addition to the cases reported above, there were 1936 cases of undulant

fever reported in the U. S. cause undetermined, however. In Canada there were 124 cases a large percentage of which were traced to milk from herds containing animals having Bang's disease.

Great progress has been made in developing safe wholesome milk supplies. In continuation of this program our efforts should be directed to:

1. Education of the consuming public to the importance of clean and healthful milk in the diet of individuals of all ages.

2. Education of dairymen to their responsibility and importance of their part in producing wholesome milk.

3. Promotion of pasteurization wherever feasible in order to bring the "greatest protection to the greatest number" of fluid milk consumers. At the same time we should not be blind to the faults of pasteurization, but should work for their correction.

4. Greater attention to the problem of safeguarding milk supplies in small communities and rural sections.

5. Recognition and encouragement of the efforts of the dairy industry in building desirable qualities into milk; such as greater nutritional values, qualities which are influenced by factors that go beyond the strict limits of hygiene and sanitation.

L.H.B.

**238. Tuberculin Testing and the Courts.** JAMES A. TOBEY, The Borden Co., New York. 25th Ann. Report of Intern. Assoc. Milk Sanitarians, p. 95, 1936.

The first court decision upholding the tuberculin test was handed down by the Supreme Court of Minnesota in 1896.

Since this first decision on tuberculin testing, the United States Supreme Court and the courts of last resorts in a number of states have sustained the validity of municipal ordinances or board of health regulations requiring the tuberculin testing of cattle and the freedom of cows from this disease.

A total of some 35 references to court decisions are cited. L.H.B.

## FEEDS AND FEEDING

**239. Biological Value of Casein as a Supplement to the Proteins of Barley in Rations for Pigs.** E. H. HUGHES, California Agr. Exp. Sta. Jour. Agr. Research, 55, 6, p. 461, Sept. 15, 1937.

It was noted that young pigs grow slowly on a ration in which barley was the sole source of protein. Feeding experiments showed that the addition of commercial casein to this ration at the rate of 1.5 per cent about doubled the growth rate and increased the gains per unit of feed consumed. However, when commercial casein was washed free of lactoflavin its addition to the ration produced no better growth than the ration in which the proteins were furnished solely by barley. Supplementing of the barley action with lactoflavin was not tried.

L.M.T.

## HERD MANAGEMENT

- 240. Shorten the Barn Feeding.** C. F. MONROE, Ohio Agr. Exp. Sta. Weekly Press Bull. 22, 51, Feb. 24, 1938.

Dairymen can shorten the barn feeding period from 1 to 2 weeks by properly fertilizing some of their permanent pasture and turning their cows out earlier.  
W.E.K.

## ICE CREAM

- 241. Which Test Gives the Most Accurate Fat Determination for Ice Cream.** FORREST C. BUTTON, Rutgers Univ., New Brunswick, N. J. J. Milk Tech. 1, 1, p. 30, Oct., 1937.

The author states that "there is still no agreement on the part of scientific workers as to the reliability of any of the modified fat tests for ice cream."

L.H.B.

- 242. Ice Milk.** F. W. MILNER. Ice Cream Field 31, 2, p. 25, 3, p. 25, July-Aug. 1937.

The author gives the results of observations on the use of ice milk in milk shakes. In attempting to arrive at the most satisfactory basis of using ice milk in such drinks he prepared milk shakes by adding the same weight of ice milk which had been frozen so as to have widely different overruns.

It was found that the use of low overrun ice milk gave higher viscosity milk shakes than the use of high overrun ice milk even though the same weights were used in both cases. Also that the volume of the prepared drink was greater when low overrun ice milk was used in its preparation than when the same weight of high overrun ice milk was employed.

The author also reports comparative costs of milk shakes made with ice milk varying in overrun.  
W.C.C.

- 243. Quality Control in the Ice Cream Plant.** H. F. JUDKINS, Sealtest System Lab., Inc., New York. 25th Ann Report of the Intern. Assoc. Milk Sanitarians, p. 290, 1936.

This paper discusses essential factors in the manufacture of high grade ice cream.

Plant, equipment, materials, manufacturing methods, personnel, etc., are included.  
L.H.B.

- 244. New Problems in Ice Cream Sanitation Committee Report.** F. W. FABIAN, 25th Ann. Report of Intern. Assoc. Milk Sanitarians, p. 330, 1936.

This is a committee report and mention is made of some of the problems

confronting the Health Official regarding counter freezers, pasteurization, and sterilization of equipment. Also fountain sanitation is discussed.

L.H.B.

Other abstracts of interest are numbers 227, 230, 234, 237, 238, 245, 253, 262, and 263.

## MILK

- 245. Investigation of the Amylase and Phosphatase Tests as an Indication of Pasteurization.** F. W. GILCREAS AND W. S. DAVIS, Div. of Lab. and Research, N. Y. State Dept. of Health, Albany, N. Y. 25th Ann. Report of Intern. Assoc. Milk Sanitarians, p. 16, Oct., 1936.

A study of the amylase test as described by Leahy made on 87 sample showed the test was accurate in 59 per cent of the cases in determining accurately the treatment received by the sample.

Conclusions were that the amylase test could not be relied on to detect accurately the degree of treatment.

The authors used and were much favorably inclined toward the Kay and Graham procedure for the phosphatase test.

L.H.B.

- 246. Homogenization as a Preventive of Oxidized Flavor.** HAROLD E. ROSS, Cornell Univ., Ithaca, N. Y. Milk Plant Mo. 26, 4, p. 36; 8, p. 40, April-May 1937.

Experimental evidence is presented showing the effectiveness of homogenization as a means of preventing the development of copper induced oxidized flavor in milk and cream. Low homogenization pressures of 500 and 1000 pounds per square inch were partially effective, but for positive results higher pressures were necessary. The development of the oxidized flavor was prevented entirely by homogenization at pressures of 1500 pounds per square inch and above. These pressures were equally effective in preventing the development of the off flavor when copper was added to the milk after homogenization.

Tables are presented giving details of all the experiments. The author advances a theory explaining in part why milk properly homogenized does not develop an oxidized flavor.

G.M.T.

- 247. The Oxidized Flavor in Milk from the Individual Cow.** C. D. DAHLE AND L. S. PALMER, Penn. Agr. Exp. Sta. Bul. 347, May, 1937.

The spontaneous oxidized flavor in milk is considered to be due to the oxidation of the phospholipid fraction of the fat globule membrane (lecithin) and the butterfat. It was determined that the enzyme-like factor responsible for off-flavor is carried in the plasma and serum portion of the milk, but that it is not responsible for the reduction of vitamin C content. The develop-

ment of oxidized flavor in milk is greatly inhibited by feeding green foods, incubation at 98° F., pasteurization above 168° F., the removal of oxygen or by the additions of vitamin C, hydroquinone, and oat flour. Pasteurization between 145–160° F. and exposure to sunlight enhance the off-flavor. Storage of susceptible samples at 40–45° F. caused a decrease in vitamin C content, but less in milks heated to 170° F. W.D.S.

- 248. Report of the Committee on Dairy Farm Methods.** F. D. HOLFORD, 25th Ann. Report of the Intern. Assoc. Milk Sanitarians, p. 303, 1936.

This report discusses information an inspector should have, also the following fundamentals: general conditions, health of cows, utensils, milking, milk house and cooling. L.H.B.

- 249. Methods of Improving Milk Supplies in Small Communities.** LESLIE C. FRANK, 25th Ann. Report of Intern. Assoc. Milk Sanitarians, p. 347, 1936.

This paper gives a very thorough report on the status of milk control in municipalities of 1000 to 10,000 population. L.H.B.

- 250. Milk Control in Small Communities on a Mandatory versus A Voluntary Basis.** C. A. ABELE, State Dept. of Health, Montgomery. 25th Ann. Report of the Intern. Assoc. Milk Sanitarians, p. 382, 1936.

Gives reasons why he thinks milk control in small communities will prove more satisfactory on a voluntary basis and that this method has several distinct advantages over the policy of mandatory compliances. L.H.B.

- 251. Experiences in Meeting Milk Flavor Problems.** C. L. ROADHOUSE, Univ. of California. 25th Ann. Report of Intern. Assoc. Milk Sanitarians, p. 201, 1936.

A discussion of normal constituents of milk influencing taste. The chloride-lactose relation is believed to be most concerned with the natural pleasant flavor of milk.

Feed flavors, rancidity, oxidized flavor, and influence of sunlight are also discussed. L.H.B.

- 252. The Influence of the Ration on Milk Flavor.** J. A. ANDERSON, Bureau of Biol. Res., Rutgers, Univ., New Brunswick, N. J. 25th Ann. Report of the Intern. Assoc. Milk Sanitarians, p. 223–238, 1936.

Observations over a period of months on two large farms producing high grade milk indicated that certain food accessories of the feed had an important bearing on the flavor development in milk after two to four days of storage. In one herd very few cows produced milk which acquired an off

flavor on storage, while in the other many cows produced such milk. Both herds received approximately the same kind of feeds, however, the one wherein the least trouble with off flavor was experienced received machine cured alfalfa hay while the other herd was fed field cured alfalfa.

Other investigators have demonstrated that machine dried alfalfa contained approximately as much carotene as did fresh alfalfa, while three days of field curing caused a loss of nine tenths of the carotene.

Substituting field cured alfalfa for machine cured alfalfa in the ration of a cow giving rancid milk had a decided effect in increasing both the intensity and frequency of flavor development. Again feeding machine cured in place of field cured alfalfa again lessened this flavor development.

Carrots (2 to 3 times richer in carotene than fresh alfalfa) were also fed with excellent results in reducing rancid flavors.

Feeds rich in carotene were also found beneficial in preventing and lessening oxidized flavor developing in milk.

Feeds rich in vitamin C (fresh cabbage) had no beneficial effect in preventing oxidized flavors.

L.H.B.

**253. The Scope of the Milk Sanitation Studies of the Public Health Service.** LESLIE C. FRANK, Office of Milk Investigations, U. S. Public Health Service, Washington, D. C. 25th Ann. Report of Intern. Assoc. Milk Sanitarians, p. 191, 1936.

During the past ten years the milk sanitation studies of the Public Health Service have included projects designed to answer the following questions:

1. How frequently do milk borne outbreaks of disease occur?

Since 1923 an annual questionnaire has been sent to health officers of all municipalities of 10,000 population and over. These surveys indicate that the average for the past 10 years is at least 43.5 milk borne outbreaks per year.

2. To what extent do American communities attempt to control milk supplies, and to what extent are their citizens protected by such major measures as pasteurization, tuberculin testing, abortion testing, etc.?

From 1927 to 1931 in municipalities of 10,000 or over, pasteurization of milk supplies increased from 81.8 per cent to 87.5 per cent. Milk from tuberculin tested cows increased from 68.1 per cent to 88.7 per cent in the same period of time.

3. How can process of pasteurization be tested to determine whether they are effective, and how can the efficiency of various types of pasteurization be compared with each other.

No apparatus should be approved that shows a temperature deviation greater than 1° F. Tests conducted on 160° F. for 15 sec. and 142° F. for 30 minutes gave assurance that either method would prevent milk borne outbreaks of disease, however, no answer is available as to which of the two methods gives the greater factor of safety.

4. How can processes of germicidal treatment of dairy and milk plant equipment be tested to determine whether they are effective, and how can the efficiency of various processes be compared?

This question is being studied. Heat methods are being compared with chemical methods. Each chlorine compound is to be tested with a test organism to determine the number of parts per million required to produce a standard percentage killing of a standard concentration in a standard time, at a standard temperature, at a standard pH, and with a standard temperature, and with a standard concentration of organic matter.

5. How should pasteurizer inlet and outlet valves be designed?

Some of the results of this study are now contained in the Public Health Service Milk Code. A more detailed publication is contemplated.

6. Are air and foam heaters necessary and how should they be designed?

Milk foam is nearly always insufficiently pasteurized. A publication on these studies is contemplated.

7. What is the cost of strictly enforcing the Public Health Service Milk Ordinance?

The mean cost reported by 74 cities which were strictly enforcing the ordinance as shown by ratings of 90 per cent or higher, was 8.3 cents per capita per year or one-half cent per gallon.

8. Does pasteurization significantly affect the food value of milk?

This study was undertaken several years ago and the results for children ten months to six years of age showed that the growth promoting capacity of milk is not significantly affected by pasteurization or other heating.

9. What is the public health significance of keeping milk cold in the home?

This study has been completed and the publication is ready for distribution.

10. Can the Public Health Service milk ordinance be successfully applied to a very large city?

During the past ten years the ordinance has been adopted by larger and larger cities. In 1935 it was adopted by Chicago and a study of the progress is being closely made there. Studies to date seem to indicate the results will be successful. If so, this should finally settle the question as to whether the Public Health Service milk ordinance is sufficiently flexible to be adapted to population groups varying in size from less than 10,000 to more than 3,000,000.

L.H.B.

**254. A Study of Milk from Apparently Normal Udders.** C. K. JOHNS, Central Exp. Farm, Ottawa, Canada. 25th Ann. Report of Intern. Assoc. Milk Sanitarians p. 145, 1936.

A study was made of heifers giving foremilk of abnormal composition from apparently normal udders.

High values for catalase, chlorides, and pH in the foremilk do not always indicate infection with mastitis streptococci or other specific pathogens.

A positive diagnosis should be based primarily on the demonstration of the causative organism. L.H.B.

**255. Milk Control in Pennsylvania.** WILBUR K. MOFFETT, State Dept. of Health, Harrisburg, Pa. 25th Ann. Report of Intern. Assoc. Milk Sanitarians, p. 165, 1936.

Pennsylvania revised its milk control law by the State Department of Health inviting the cooperation of the local health officers, the milk dealers, the ice cream manufacturers, the medical associations and any other organizations which were interested in health measures. We tried to take the best ideas from those used in New York, New Jersey and other states and incorporated them in the Pennsylvania regulations, known as Act 210, which was signed by the Governor, July 2, 1935.

We still have a system of approved inspectors but standards for these inspectors have been raised. There are about 350 of these approved inspectors and they are hired by the plants. To obtain a certificate as an approved inspector, they must have some technical training, some practical training, a lot of common sense, and good moral character, besides passing an examination which is not given out in advance. These men are supervised by 28 state inspectors.

Before milk, or any dairy product, or ice cream or any product that goes into ice cream can be sold in Pennsylvania, the dealer, at his own expense, must put in shape his own supply to meet the requirements of the state. After that is done, he makes an application. The state then sends men to check on the work of the approved inspector, and in case of new applications to do business in the state it means a 100 per cent check on every farm that is shipping to that plant. That means that all butter used in ice cream must come from an inspected source. Also all evaporated milk used in Pennsylvania must come from an inspected source.

All raw milk producers are inspected by the Department of Health and not by approved inspectors. L.H.B.

**256. The Use of Resazurin in Determining the Bacterial Quality of Milk and Cream.** J. N. WARNER, Iowa State College, Ames, Iowa. Dairy World 16, 9, p. 18, Feb., 1938.

In a comparison of the resazurin test with the methylene blue test, utilizing methylene blue chloride and methylene blue thiocyanate and reading the resazurin tests at the violet, pink and white stages, the following conclusions were drawn. The resazurin test offers no advantage over the methylene blue if resazurin-white is used as the end point. The resazurin-pink and resazurin-violet reduction times are considerably shorter than the methylene blue but classification of samples does not parallel the latter test. Whether the difference would result in a more desirable classification or a less desirable classi-

fication is not shown in the data obtained. The resazurin test showed no advantage over the methylene blue test for use with pasteurized milk or cream or ice cream mix.  
F.J.D.

**257. Some Factors Affecting the Accuracy of the Babcock Test on Composite Samples of Milk.** C. W. ENGLAND AND G. D. D'AMBROGI, Univ. of Maryland, Agr. Exp. Sta., College Park, Md. Bull. 413, Oct., 1937.

Composite samples of milk were prepared by making daily additions of 10.4 ml. each. Those samples were held for seven, ten and fifteen days at 45°, 60°, 80° and 100° F. and were preserved by one mercuric chloride tablet (0.48 gram tablet containing 46.66 per cent  $\text{HgCl}_2$ ). Composite samples were also prepared with one-half, one and two mercuric chloride tablets and held at 60° and 100° F. for seven, ten and fifteen days. Fresh samples were tested daily and their average test compared with the tests of all composite samples.

Samples of milk were brought to a definite volume by adding 156 ml. to each sample bottle, the sample then being tested immediately for fat content. These samples were held at 45°, 60°, 80° and 100° F. for seven, ten, and fifteen days, and were preserved by one mercuric chloride tablet. Samples brought to a constant volume were also preserved with one-half, one, and two mercuric chloride tablets and held at 60° and 100° F. for seven, ten, and fifteen days. The fresh sample tests were compared with the tests of all preserved samples.

All tests were made by the Babcock Method. A summary of results follows:

1. The fat tests on all composite and preserved sample, regardless of the length of time held, averaged lower than the fresh sample average test. As the time of holding is increased the resulting fat test is decreased.

2. The fat tests on all composite and preserved samples, regardless of the temperature held, averaged lower than the fresh sample average test. As the temperature of holding is increased, the resulting fat test is decreased.

3. Samples preserved by one mercuric chloride tablet gave the highest, one-half tablet the next highest, and two tablets the lowest fat tests. This holds true for all periods of time at both 60° and 100° F. Regardless of the amount of mercuric chloride used, all tests on composite and preserved samples were lower than the fresh sample test.

The average of all fresh sample tests was 4.355 per cent. The average test of all composite and preserved samples held for seven and ten days at 45° and 60° F. using one mercuric chloride tablet was 4.302 per cent. Thus it may be stated that when preserved samples were held under excellent conditions, the fat test averaged 0.053 per cent lower than the true fresh sample test.

The average test of all composite and preserved samples held for fifteen

days at 45° and 60° F. using one mercuric chloride tablet was 4.275 per cent, or 0.080 per cent lower than the true fresh sample average.

The average test of all composite and preserved samples held at 80° and 100° F. using the mercuric chloride tablet was 4.188 per cent, or 0.167 per cent lower than the true fresh sample average. C.W.E.

258. **Salvaging Return Milk.** ANONYMOUS. Milk Dealer 27, 4, p. 44, Jan. 1938.

A brief summary of how some dealers, in localities which permit milk to be returned to the routes the second day, are eliminating the loss on returns by the use of refrigerated delivery trucks. A cross section of replies received from various sections of the country regarding the use of returns is also given. C.J.B.

259. **Report of the Committee on the Food Value of Milk and Milk Products.** G. C. SUPPLEE, Bainbridge, N. Y. J. Milk Tech, 1, 1, p. 16, Oct. 1937.

A brief summary of the findings of various investigators on factors effecting the food value of milk. L.H.B.

260. **Engineering of Pasteurization.** C. A. HOLMQUIST AND W. D. TIEDERMAN, State Dept. of Health, Albany, N. Y. J. of Milk Tech. 1, 1, p. 11, Oct. 1937.

Some of the faults of old type pasteurizers are cited, and how these have been eliminated by dairy engineers in testing, and redesigning equipment so that equipment is now available that will pasteurize milk with a full factor of safety. L.H.B.

261. **The Resazurin Test—Preliminary Studies on Its Practicalities and Possibilities.** J. A. KEENAN, W. D. BARRETT, AND H. RUTAN, Whiting Milk Co., Boston, Mass. J. of Milk Tech. 1, 1, p. 22, Oct., 1937.

The authors concluded that the resazurin test will impart more information concerning the quality of milk in one hour's incubation than will the methylene blue test in six hours' incubation.

The test is more sensitive to abnormal milks (colostrum and mastitis milks) than is the methylene blue test. L.H.B.

Other abstracts of interest are numbers, 225, 230, 233, 234, 235, 236, 237, 238, 262, 263, and 264.

### MISCELLANEOUS

262. **Report of Committee on Milk Plant Practice.** A. R. TOLLAND, 25th Ann. Report of Intern. Assoc. Milk Sanitarians, p. 181, 1936.

With but one exception all members of the committee replying on the question of bottling orange juice in milk plants the practice should be forbidden. A number of states and cities require that orange drinks be processed in equipment (or rooms) not used for handling fluid milk.

Reconstructed dairy orange beverages rapidly lost their vitamin C content on standing at room temperature. The loss at cold storage temperature is much less but is still considerable. L.H.B.

**263. Vitamin C Content of Dairy Orange Beverages.** M. J. MACK, C. R. FELLERS, W. A. MACLINN AND D. A. DEAN, Mass. Agr. Exp. Sta., Amherst, Mass. 25th Ann. Report of the Intern. Assoc. of Milk Sanitarians, p. 267, 1936.

Chemical methods for determining vitamin C content of orange beverages were found to agree closely with the biological assay method.

Twelve samples of ten different dairy orange beverages were found to contain from 0.2 to 53.0 units of vitamin C per ounce.

Fresh orange juice contained 228 to 258 units per ounce; while canned orange juice had slightly over 200 units per ounce.

Reconstituted dairy orange beverages lose their vitamin C very rapidly at room temperature, usually about 60 per cent in 20 hrs. At 40° F. the loss was 15 per cent or more in the same time. In two days the loss was even greater; yet many dairies do not make up the product daily. L.N.B.

**264. The Physician and Our Daily Bread.** American Institute of Baking, 9 Rockefeller Plaza, New York, N. Y. Pp. 19, 1938.

Although this booklet is devoted to the subject of bread and its proper place in normal and reducing diets, there are many allusions to milk in it. Thus, it is stated that practically all white bread now contains at least 6 per cent skim milk solids, which enhance the nutritive properties of the loaf by adding proteins, minerals, and certain vitamins. Several pages of sample daily menus for use in reducing diets are prefaced by the statement that "a pint of certified or pasteurized milk, or its equivalent in other dairy products, is desirable in all daily reducing diets." Bread is recommended in these diets as a necessary source of the carbohydrate needed for the most efficient burning of body fat. This pamphlet is accepted by the Council on Foods of the American Medical Association. J.A.T.

Another abstract of interest is number 235.

## PHYSIOLOGY

**265. Fat Feeding and Cholesterol Absorption.** ROBERT PERCIVAL COOK, Biochem. Lab., Cambridge, England. *Biochem. J.* 31, 410, 1937.

In a previous study it was observed that with growing rats cholesterol is absorbed only in the presence of free fat in the diet, a cholesterol "fatty" liver being induced solely under these conditions. In this study the experiments were conducted to determine the effect of the amount of fat on cholesterol absorption. Growing rats were fed on diets containing 15, 20 and 30 per cent fat (arachis oil) with and without 2 per cent cholesterol. Cholesterol was observed to have a deleterious effect on the growth rate, which was most marked with the 15 per cent fat diet. This affect is, during the first few weeks probably due to the reduced food intake of the animals, after which period the rats adapt themselves to the ration. The absorption of cholesterol is not increased by raising the fat concentration in the diet. Approximately 30 per cent of the cholesterol fed remains unaccounted for, as determined by unsaponifiable fractionation of the faeces and the animals' bodies.

K.G.W.

266. A Study of the Effect of Overfeeding on the Protein Metabolism of Man. I. The Effect of Superimposing Raw and Boiled Milks on a Diet Adequate for Maintenance. II. The Superimposition, on a Diet Adequate for Maintenance, of Beef (or Soya Flour) Plus Lactose Plus Butter, Equivalent in Protein, Carbohydrate and Fat Content to a Liter of Milk. D. P. CUTHBERTSON, ALEXANDER McCUTCHEON AND H. N. MUNRO, Institute of Physiology, Univ. of Glasgow, Scotland. *Biochem. J.* 31, 681, 1937.

Seven subjects of good physique, members of the teaching or laboratory staff, were given self-selected basal diets including 500 ml. raw milk, with a constant water intake. After the nitrogen equilibrium had been determined for each of the subjects, the diet was superimposed by one liter of either raw or boiled milk. This was conducted both in one day and 8 day tests. Superimposition of a liter of either raw or boiled milk on the diet, adequate for maintenance of body weight and nitrogen equilibrium in the adult human subject, caused an increase in body weight and a marked retention of nitrogen and sulphur but not of calcium. No significant difference could be observed in the metabolic fates of the proteins of raw and boiled milks. When sodium caseinate equivalent in nitrogen to the added liter of milk was substituted in the diet of one subject, the retention of nitrogen was definite, but not of the same magnitude as for milk.

The superimposition of beef plus lactose plus butter equivalent in protein, carbohydrate and fat content to a liter of milk also effected a definite saving of food nitrogen. Soya flour plus lactose plus butter gave similar results.

K.G.W.

# JOURNAL OF DAIRY SCIENCE

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National Institute for Research in Dairying, Reading, England	United States Department of Agriculture
New York Association of Dairy and Milk Inspectors	

# ABSTRACTS OF LITERATURE

## CHEMISTRY

- 267. A New Method for the Determination of Butterfat in Dairy Products.** JOHN GOLDING. *J. Dairy Research* 8, p. 275, 1937.

A simple gravimetric method suitable for the determination of fat in cream, ice cream mix, milk, and possibly other dairy products is described. The fat is separated by churning following the addition of a variable quantity, dependent on the product being tested, of a reagent consisting of 75 ml. of C. P. ammonium hydroxide, 35 ml. of n-butyl alcohol, and 15 ml. of 95 per cent ethyl alcohol. The butter is washed with water and then removed to a metal dish for frying. The percentage of fat is calculated from the weight of dried fat and the weight of the original sample. Results were found to agree with the Roesse-Gottlieb analysis within  $\pm 0.05$  per cwt.

S.T.C.

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

Abstracts of interest are numbers 267 and 271.

## DISEASES

- 268. Bovine Mastitis. III. A Comparison of the Bacteriological Reactions of Normal and Mastitis Milk from Young Cows.** RALPH B. LITTLE, The Rockefeller Inst. for Med. Research, Princeton, N. J. *Cornell Veterinarian* 28, 1, p. 23, Jan., 1938.

The milk from eight first calf heifers was examined before and after their udders were infected with a double zone hemolytic streptococcus. The methods used were daily examinations of chlorine, leucocyte count, bacteriological examination of the milk, and pH values. After the onset of subclinical mastitis in these eight animals, 2163 daily examinations of the fore milk showed that the bacteriological plating of the milk in blood agar was more efficient in the detection of infection than any other method employed for typical hemolytic streptococci were observed in the milk of every examination. The next more reliable test was the direct leucocyte count; in 94 per cent of the examinations the count per cc. of milk was between 300,000 and 10,000,000 cells or over.

L.A.M.

- 269. Detection of Mastitis by the Bromthymol Blue Test, Leucocyte Count, and the Microscopic Examination of Incubated Milk.** A. C. FAY, H. W. CAVE AND F. W. ATKESON, Kan. Agr. Exp. Sta., Manhattan, Kan. *Cornell Veterinarian* 28, 1, p. 40, Jan., 1938.

On a basis of routine examination of individual quarter samples of milk from 114 cows in the college herd the animals were segregated into three

principal classes: Class A, 70 animals regarded as free from mastitis; Class B, 17 head regarded as suspicious for mastitis because of high leucocyte count (500,000 or more per cc.) in the milk from one or more quarters; Class C, 27 head, regarded as positive for mastitis because of presence of long chained streptococci in samples incubated 16 hours and usually though not necessarily a high leucocyte count. The results of the studies showed that although the Bromthymol Blue test rarely gives a false reaction with a known negative cow, it fails to detect a sufficiently high percentage of the opposite cows to recommend it as a sole means of identification of mastitis for segregation purposes. The fact that high leucocyte counts above the arbitrary standard of 500,000 per cc. were found in 36.7 per cent of the samples containing long chained streptococci suggests that the standard is too high for proper interpretation. Leucocyte counts above 100,000 per cc. and the appearance of streptococci in incubated samples of milk in chains of only medium length frequently gave fore warning of impending mastitis. It was found inadvisable to move a cow from an infected group to the non-infected group because of a few negative tests. L.A.M.

**270. Infectious Bovine Mastitis. 5. Bovine Mastitis and Milk Yield.**

G. C. WHITE, E. O. ANDERSON, R. E. JOHNSON, W. N. PLASTRIDGE, F. J. WEIRETHER, Storrs Agr. Exp. Sta., Storrs, Conn. Bul. 220, August, 1937.

To determine the effect of mastitis on milk yield data are studied on 35 cows in the Connecticut State College dairy herd and on 22 cows in a farmer-owned herd. Since in both herds animals with acute cases were discarded, the study deals chiefly with incipient and mild forms of the disease.

Four tests are used to determine the status of animals during the various lactations, namely: identification of organisms, bromthymol blue test, leucocyte count, and sediment test.

Lactations covering 240 days corrected to full age and to three time milking for the college herd and two time milking for the farmer's herd, are used to compare production before and after reaction. In the college herd a loss of 679 pounds of milk or 6.5% and in the farmer's herd, of 559 pounds of milk or 5.7% resulted from infection. The amount of loss increased with the number of quarters affected, reaching 1134 pounds or 12.0% and 1111 pounds or 11.2% respectively in the college and the farmer's herd, when all four quarters reacted positively.

Individual lactation period curves covering four or more lactations are shown for five animals and compared with the normal expected production curve.

About three-fourths of all animals show a loss in yield following reaction. Some individuals may pass through several lactations before yield is drastically reduced, or the milk becomes abnormal in appearance; some show a

loss for one or two lactations, and then recover in yield; others suffer a drastic loss in yield and may continually produce abnormal appearing milk. The evidence shows no shortening of the lactation period.

No evidence was found to indicate that mastitis decreases the percentage of fat in the milk, the average tests being 4.16 before and 4.15 following reaction.

A.I.M.

## FEEDS AND FEEDING

**271. The Relative Values of Raw and Pasteurized Milk in the Feeding of Calves.** J. WILKIE, S. J. EDWARDS, A. B. FOWLER AND N. C. WRIGHT. *J. Dairy Research* 8, p. 311, 1937.

Bull calves from tuberculin-tested Ayrshire herds were fed on raw or commercially pasteurized milk up to 12 weeks of age, in amounts in relation to their body weights. This diet was supplemented from the eighth week by hay at the rate of 2/3 pound per head per day. The milk used was mixed milk from untested herds, the raw and pasteurized milk being derived from the same bulk samples.

No significant differences were noted in gain in live weight or in skeletal growth. Marks awarded by experimental stock judges showed consistent differences in favor of the pasteurized milk-fed group.

Inoculations of grouped daily aliquots of raw milk twice weekly into duplicate guinea-pigs resulted in finding viable tubercle bacilli in 70 per cent of the samples and *Brucella abortus* in 38 per cent of them. The pasteurized milk samples were uniformly negative to both tests. The differences in tuberculous infection of the two types of milk were reflected in the results of tuberculin tests and post-mortem examinations on the calves. Twenty-four out of thirty-six calves fed on raw milk reacted to the test. One calf in the pasteurized milk-fed group reacted to the test, but post-mortem examination and inoculation of glandular material into guinea-pigs failed to confirm the presence of any tuberculosis.

S.T.C.

**272. Further Studies on the Influence of Green Fodder, Silage and Hay on the Metabolism of Ruminants.** FRITZ SCHNEPF, Tierzucht-institut der Albertus-Universität Königsberg. *Biedermann's Zentralblatt, Abteilung B: Tierernahrung*. 9, 3, p. 191, 1937.

These studies were planned to determine (1) the relationship between Ca metabolism and pH of the urine on one hand, and CO<sub>2</sub> tension of the blood plasma and pH of the blood on the other hand, and (2) the effect on the animal body of green feeds preserved in different ways.

Wethers were fed a mixture of second cutting red clover and timothy as green plants, as hay, and as silage. The hay was dried on racks. To the silage was added as a preservative for each 100 kg. of green crop (1) ten acid equivalents of HCl, (2) ten acid equivalents of H<sub>2</sub>SO<sub>4</sub>, (3) one per cent of sugar.

Results indicate that;

(1)  $\text{CO}_2$  tension is as good a criterion of the potential alkalinity or acidity of a feed as is the Ca metabolism; the pH of the blood is too constant to be of any value for indicating small differences; the pH of the urine is influenced markedly by the nature of the ration, but individual differences are often greater than species differences.

(2) Hay has a stronger basic effect than green plants.

(3) Silage preserved with sugar has about the same effect on acid-alkali balance in the body as hay or green plants.

(4) Silage preserved with HCl or  $\text{H}_2\text{SO}_4$  exerts a distinctly negative effect on Ca metabolism and the blood picture.

(5) If about one-fourth of the dry matter is in the form of hay, sometimes restoration of balance is noted, but it is not enough to compensate the total effect of the mineral acids.

J.G.A.

**273. Utilization Experiments on Ruminants with Artificially Dried, Chopped Protein-Rich Green Fodders.** G. FRÖLICH UND F. HARING, Institut für Tierzucht und Molkereiweisen der Martin-Luther-Universität Halle a.S. Biedermann's Zentralblatt, Abteilung B. Tierernährung. 9, 3, p. 204, 1937.

Digestibility by wethers of the nutrients of three dried products prepared from green alfalfa and from vetch mixture (so-called Landsberg mixture) by the Rema Rosin drying method, is reported.

Digestibility of artificially dried green alfalfa was improved by grinding as compared with chopping. The chopped vetch mixture was somewhat more digestible than the dried alfalfa. The following values are reported:

Digestible crude protein—in alfalfa meal, 10.83%; in chopped alfalfa, 10.35%; in chopped vetch, 10.17%; starch values—29.33, 26.90, and 38.52% respectively.

J.G.A.

**274. Significance of Cod Liver Oil in Calf Feeding.** LAURI PALOHEIMO, Institut für Haustierlehre der Universität Helsinki. Biedermann's Zentralblatt, Abteilung B. Tierernährung. 9, 3, p. 234, 1937.

In an earlier paper (this journal, 9, 52) an account was given of work in which Ayrshire calves were reared with small quantities of whole milk, their requirements for fat-soluble vitamins being supplied by addition of cod liver oil.

The work has been continued, cod liver oil being omitted, other conditions being the same as already noted. Maximum gains during the first few months were not striven for but the calves were well cared for, and were in good health throughout. Indigestion was specially guarded against.

Under these conditions, even when the total quantity of whole milk was limited to 10–15 kg., calves seemed to thrive nearly as well as when 5–10

grams of cod liver oil were fed daily. The dams, with two exceptions, had received feeds rich in vitamins during the latter part of the gestation period. But the two exceptions indicate (but two calves are too few to prove) that this preparatory feeding of the dams is not absolutely necessary for thrifty calves, even if these are reared with very little whole milk and without cod liver oil.

J.G.A.

**275. Studies on the Influence of Air Tight Covers on the Preservation and Value of Silage.** KURT DIETRICH, Tierzucht-Institut der Albertus Universität Königsberg. Biedermann's Zentralblatt, Abteilung B: Tierernährung. 9, 3, p. 255, 1937.

Glass cylinders were filled with chopped clover or marrow stem cabbage. To one lot no preservative was added; others had sugar or sulfuric acid added. The cylinders were closed with rubber stoppers each provided with a fermentation tube. A parallel series were sealed with a layer of loam. Larger scale trials were conducted in Aurich fermentation vessels of 1 cubic meter capacity, or in Tschechnitz fermentation chambers.

Conclusions reached were:

1. Complete exclusion of air hinders fermentation, as indicated by high pH values, very little lactic acid formation, a large amount of combined acetic acid, and formation of a small amount of butyric acid.
2. Wilting before ensiling still further slows up the process with clover; with m.s. cabbage more compact storage and corresponding improvement in the silage was noted.
3. Sugar favorably affects the ensiling of fresh plants as well as of wilted ones.
4. Chopping effected an improvement in all cases.
5. Addition of  $\text{CO}_2$  has a favorable effect, but this is small in comparison with the effect of sealing the chamber with a layer of loam.
6. Addition of mineral acids checks the formation of  $\text{CO}_2$  almost completely.
7. Comparison of the silage from air tight vessels with that from similar vessels covered with a layer of loam indicates that in all cases the upper layers were superior when the latter method was used.
8. There were no considerable differences in protein degradation in the several lots.
9. Digestion trials (wethers) do not indicate any superiority of one method over another.

J.G.A.

## HERD MANAGEMENT

**276. Biometrical Study of the Production Improvement in a British Friesian Herd.** HANS LÖRTSCHER, Zeitschr. Züchtung. Reihe B. Tierzücht. u. Züchtungsbiol. 39, 3, p. 257, 23 fig., 1937.

The milk production records from 1906 to 1932 in a large Friesian herd in England were studied to measure the non-hereditary factors influencing milk yield and to find ways to correct for those factors when studying inheritance or making selections. About 4000 lactations from some 1200 cows were included. A multiple regression equation and nomogram were developed for standardizing the test-year records for age of the cow when the test year began, for length of the dry period, for length of the service period, and for the inter-relations between these factors. The length of the preceding dry period was of slight importance unless it was shorter than three weeks. The month of calving, the sex of the calf, and whether it was a twin or a single birth had little effect. Expressing each individual yield relative to the herd average for the same year (the "Stalldurchschnitt" method of Peters, v. Patow, Krüger, and others) was studied as a means of correcting for intangible environmental factors such as weather, changes in feeding policy, etc. Complete reliance on these relative figures would imply that all year-to-year differences in the herd average are of environmental origin. On the contrary the author finds that in his material many of the differences in the yearly averages resulted from changes in the average genetic composition of the herd. This conclusion is based on the steadiness of the trends and on comparing the year-to-year changes of the herd average when only those cows which had records in both years were included, with the contemporary changes in the herd average when it included the records of all cows. In this procedure the author offers a way to use the herd average to correct for unrecorded changes in herd environment, yet without assuming that the average genetic composition of the herd is unchanging. That the method will also furnish a bridge for comparing the records of two cows in different herds unless those two herds happen to have interchanged a number of cows, is not apparent.

The author concludes that 50 to 70 per cent of the variance in the milk records with which he worked (corrected for age, dry period and service period) is genetic, but that construes as genetic nearly all of the differences in yearly averages. Those differences account for nearly 24 per cent of the total variance in individual records. Regression of individual productions on changing conditions is thought not to be entirely linear and not to follow the same course for all cows. This makes Mendelian analysis difficult, if possible at all.

This particular herd was outbred in its early history, the breeding stock coming from various other British Friesian herds; then it was outbred with stock imported from Holland; finally there was a period of breeding entirely within the herd, with linebreeding directed toward the imported animals but with efforts to avoid inbreeding while staying within the herd. The inbreeding did not become high enough to produce noticeable effects. Coefficients

of inbreeding and *inter se* relationship are presented to make the description of the breeding policy of the herd more complete and quantitative.

J.L.L.

**277. Measuring Milk Producing Ability.** L. KRÜGER. Breslau. Züchtungskunde 13, 1, p. 2, Jan., 1938.

The author emphasizes the distinction between the milk producing *ability* of a cow and the *record* of the milk she actually produced. His method of correcting for differences in environmental circumstances is presented and its use is illustrated with a few examples. Yields are corrected to a standard interval of 360 days between calvings. The last 50 days of this interval are a dry period. Correction is also made for deviations in the length of the preceding calving interval and dry period and for age. Corrections for unrecorded environmental conditions applying to that herd are made by multiplying the record of this cow by the ratio of the standard production in that region for that system of dairying to the average production of the cows which freshened in the same herd during the six months centering around the time that this cow freshened. The inherent assumption, that the average producing ability of the group of cows freshening in those six months in that herd is equal to the average producing ability of all cows of that region, doubtless has exceptions and needs some qualifications. If those can be had, this process of correction offers an automatic way of correcting for general conditions of management and feeding, without recording what those were nor estimating the effect of each. There is, of course, need for judgment in deciding what groups of herds should be combined to provide the standard for that region and for that type of agriculture. There is also opportunity to exercise judgment in discarding records which were "abnormal" for reason of accident or distinct and unmistakable illness. J.L.L.

## ICE CREAM

**278. A Summary of Six Years of Research on Tallowy Flavor in Strawberry Ice Cream.** C. A. IVERSON, Iowa State College, Ames, Iowa. Proc. 37th Ann. Conv. Intern. Assoc. Ice Cream Mfgs. 2, p. 7, Oct., 1937.

The author concludes from this study that oxidases from the fruit are not responsible for causing oxidized flavors in strawberry ice cream.

The higher the iron content of strawberry ice cream, the less oxidized flavor developed. This was thought due to the existence of ferrous iron in combination with a milk constituent, serving in this combination as an anti-oxidative catalyst.

Reichart-Meissl numbers and acetyl values determined on fat extracted from ice cream showed no relation to the rate of development of oxidized

flavors. The trend in the iodine number changes indicated fat oxidation during development of oxidized flavors.

Oxidized flavors developed more frequently when whole or skim condensed milk were used in mixes as compared with dry skim milk or condensed skim or whole milk made in stainless steel pans.

The author concludes, further, from experimental evidence that the addition of fruits to ice cream actually retards the development of oxidized flavors in ice cream.

The copper content of mixes in which no oxidized flavors developed was found to be less than 1.18 p.p.m., whereas the lower limit of the copper content of ice creams which became oxidized was 1.80 p.p.m. When mixes contained copper contents between these limits and developed oxidized flavors, it was assumed that factors other than the copper content contributed to the development of the off-flavor. It is also stated that the gross quantity of copper present is not the only factor of importance, but the state in which the copper exists in the ice cream is also of significance. M.J.M.

**279. Quality Through Freshness.** A Discussion led by H. F. JUDKINS, National Dairy Prod. Corp., New York, N. Y. Proc. 37th Ann. Conv. Intern. Assoc. Ice Cream Mfgs. 2, p. 104, Oct., 1937.

(a) A. C. BITTER, Wm. Neilson, Ltd., Toronto, Can.

Packages are marked in code and each driver is equipped with a key. It is his responsibility to see that the oldest stock is sold first in the stops along his route. By limiting the number of articles and eliminating slow selling products in winter months it was possible to carry only five standard bricks and a weekly special. By so doing, fresher ice cream has been sold and sales have increased considerably.

(b) J. A. CLUTTER, Dairyland, San Antonio, Texas.

In each retail store some ice cream was set aside, held for three weeks, and then scored against the fresh product. On the average, the ice cream had deteriorated about two points in flavor score over the storage period of three weeks. If fresh high quality ingredients are used to make a desirable product, it should be marketed promptly before the ice cream deteriorates in quality.

(c) GEORGE A. KURK, Beatrice Creamery Co., Lincoln, Neb.

The necessity of selling fresh ice cream has been shown repeatedly. In order to achieve this, careful supervision of the hardening room is essential, a survey of the dealers' sales possibilities is desirable, and lastly many companies should eliminate small accounts where the daily sales are low and the ice cream deteriorates before being sold.

(d) R. J. QUIRRE, United Farmers Cooperative Creamery Assn., Charlestown, Mass.

The consumer often does not get the same high quality ice cream which is placed in the cabinet of the distributor. The answer lies in what might be called sales or service contact work which can be done by the route salesman, the territory salesman or one especially designated for the work. The distributor's stock should be checked, the mechanical and sanitary character of the cabinet should be watched and the cabinet should be operated at a sufficiently low temperature for packaged goods.

(e) J. FRANK WARD, *Midwestern Dairy Products*, Salt Lake City, Utah.

Much of the trouble with off-flavors is due to the use of inferior products as well as the failure to market ice cream promptly. Both factors must be watched.

The operation of retail ice cream stores by ice cream manufacturers has done much to teach the manufacturer the kind of ice cream the public prefers.

#### SUMMARY BY MR. JUDKINS

Data were presented to show the variance in rate of turnover for one dealer as compared to another. Bulk sales varied from 2 to 39 gallons per gallon of inventory for the period of October 1 to April 1, 1934. The variation in sales of brick ice cream was given for the same period, as well as the distribution of products stocked in the cabinets. From this information the following recommendations were made:

1. The sales department should study the relation of sales volume to flavors of bulk and package goods carried.
2. Brick package units such as one-half gallon units, should be kept in the cabinet properly.
3. Drivers should be instructed regarding the proper use of these units and should educate the dealers how to use them. M.J.M.

**280. The Statistical and Accounting Bureau.** O'NEAL M. JOHNSON, Intern. Assoc. Ice Cream Mfgs., Washington, D. C. Proc. 37th Ann. Conv. Intern. Assoc. Ice Cream Mfgs. 3, p. 7, Oct., 1937.

During the year the Statistical and Accounting Bureau released three bulletins on taxation. The Association Accounting system was installed in a considerable number of plants. Other activities of the bureau included a report of the survey of the retail store, the advertising analysis and the analysis of ice cream sales, and the ice cream sales index for 1936. Preliminary reports of sales in 1937 have also been released. M.J.M.

**281. The Accountant Looks Toward 1938.** J. S. BLOOM, *Abbotts Dairies, Inc.*, Philadelphia, Pa. Proc. 37th Ann. Conv. Intern. Assoc. Ice Cream Mfgs. 3, p. 10, Oct., 1937.

It is the accountant's job to ascertain the trend in cost of raw products, manufacturing and labor costs, taxes, sales, new demands in sanitation and the trend in other costs as well. It is necessary to chart the course of busi-

ness if comptrollers and accountants are to serve the best purposes of the industry. M.J.M.

**282. Controlling Advertising Costs.** K. R. LEACH, Dairymen's League, Inc., Syracuse, N. Y. Proc. 37th Ann. Conv. Intern. Assoc. Ice Cream Mfgs. 3, p. 16, Oct., 1937.

The proper control of advertising costs depends upon :

1. The setup of a budget and advertising plant at the beginning of the year.
2. The adherence to this budget and plan during the year.
3. The constant checking of the effectiveness of advertising both from an expense and a result point of view.
4. Proper checking of expenditures as to accuracy of charges made against the advertising account.
5. A preparation of analysis for study by management to enable management to plan its future advertising programs.

These five things should form a basis for proper control of advertising and should increase the effectiveness of the advertising. M.J.M.

**283. Controlling Selling Costs.** H. W. BRIGHAM, Teall's Ice Cream Co., Rochester, N. Y. Proc. 37th Ann. Conv. Intern. Assoc. Ice Cream Mfgs. 3, p. 22, Oct., 1937.

The control of selling costs is more or less controlled by the policy set by the management. It is possible in the larger company with a large sales force to consolidate territories and thus save expense. However, the company with three or four men traveling finds it almost impossible to vary the budget for sales expenses without altering the effectiveness of the work.

M.J.M.

**284. Controlling Trucking Costs.** H. W. SCHYELKE, Southwest Ice and Dairy Products, Oklahoma City, Okla. Proc. 37th Ann. Conv. Intern. Assoc. Ice Cream Mfgs. 3, p. 30, Oct., 1937.

Selling and delivery expenses in many businesses have been steadily mounting. The qualifications of the driver salesman affect the trucking costs on his route. The type of delivery service and the kind of delivery equipment enter materially into determining trucking costs. Telephone expenses and special delivery service often are allowed to increase costs unnecessarily.

The truck salesman should keep a detailed expense account and the adherence to this record keeping often helps to show him the necessity to keep costs lower when possible. M.J.M.

**285. Controlling Cabinet Costs.** J. E. SHIPLEY, Abbotts Dairies, Inc., Philadelphia, Pa. Proc. 37th Ann. Conv. Intern. Assoc. Ice Cream Mfgs. 3, p. 34, Oct., 1937.

By keeping simple monthly accounts of cabinet service work done, repair shop overhead, truck costs, and expenditures for materials, it is easy to determine cabinet service costs. With this information it is possible to decide whether it would be cheaper to hire an outside cabinet service contractor. The data should also show whether it is cheaper to rebuild or to buy new equipment; or if repairs increase, the real cause of the increase can be determined. The need for having adequate records so that the causes for changes in costs can be determined is imperative. M.J.M.

**286. What Management May Expect from the Association Accounting System.** C. A. ARMITAGE, United Farmers' Coop. Creamery Assn., Charlestown, Mass. Proc. 37th Ann. Conv. Int. Assn. of Ice Cream Mfgs. 3, p. 42, Oct., 1937.

There are many services one should expect from the association's accounting system. It should show when new equipment should be bought, what the operating costs should be, and what savings should be made. The system shows fluctuations in expenses and furnishes a basis for the investigation of these expenses. Such a study should be made at regular intervals. The system provides a basis for determining the costs of products and production and in this way makes it possible to ascertain the necessary selling price for each item. By showing which items are most profitable the accounting system shows which products to feature.

Intelligent merchandising is impossible without knowing unit costs for each product made. The association accounting system furnishes this information. M.J.M.

**287. Proved System Short Cuts.** O'NEAL M. JOHNSON, Intern. Assoc. Ice Cream Mfgs., Washington, D. C. Proc. 37th Ann. Conv. Intern. Assoc. Ice Cream Mfgs. 3, p. 47, Oct., 1937.

A number of changes and additions to the Association Accounting System which have increased the effectiveness of the system were explained. This was followed by a discussion of problems and suggestions pertaining to accounting. M.J.M.

**288. Cutting Office Costs.** J. S. BLOOM, Abbotts Dairies, Inc., Philadelphia, Pa. Proc. 37th Ann. Conv. Intern. Assoc. Ice Cream Mfgs. 3, p. 65, Oct., 1937.

Since the main expense in office costs is labor, the main problem in controlling the costs is to study labor costs. There are different grades of labor to be done in an office and a person trained and fitted for each grade should be employed. A program of work should be planned for each person in the office.

Other important considerations are to have the necessary mechanical appliances for most efficient work from the employees of the office, and the

office should be so organized that the flow of work from person to person is accomplished efficiently.  
M.J.M.

- 289. Late Developments in Hardening Room Installations: Bay Type of Vertical Coils.** RIDGWAY KENNEDY, JR., Abbotts Dairies, Inc., Philadelphia, Pa. Proc. 37th Ann. Conv. Intern. Assoc. Ice Cream Mfgs. 2, p. 20, Oct., 1937.

The ammonia coils used in the hardening room were installed in a vertical position in bays or bins and the ice cream was stacked in the bays. A coil unit was developed which could be fabricated and welded at the factory. Each bay was of the same size and 175, 2½ gallon cans were stacked in each bay. The article contains photographs and figures and complete instructions for the construction of this type of hardening room.

There are several advantages of the bay type of installation. Defrosting proved unnecessary because the frost was shaken or rubbed off the coils by piling the cans of ice cream against the coils. The amount of pipe in the room was reduced from forty thousand feet of two-inch pipe to twenty-five thousand feet of one and a quarter inch pipe. The refrigeration is utilized more efficiently. Less difficulty from ammonia leaks has been experienced.

M.J.M.

- 290. Problems in Meeting Bacterial Standards for Ice Cream.** H. MACY, Univ. of Minnesota, St. Paul, Minn. Proc. 37th Ann. Conv. Intern. Assoc. Ice Cream Mfgs. 2, p. 36, Oct., 1937.

Sanitary codes adopted by most states have bacterial standards which range between 100,000 and 500,000 per millileter. However, certain cities have adopted maximum bacterial standards as low as 25,000 per millileter. Considerable vigilance is necessary to meet such exacting requirements.

The problems of meeting bacterial standards involves three issues, namely, the procurement of high quality ingredients, the effective pasteurization of the mix, and the maintenance of proper sanitary conditions within the plant at all times. When these issues are satisfactorily met, the most exacting standards may be regularly observed.

An efficient laboratory service is essential if bacterial counts are to be controlled successfully. Constant checking of materials, plant processes and sanitary measures is essential. If the cost of such service seems prohibitive for the smaller companies, thought should be given to the formation of cooperative laboratory service.

M.J.M.

- 291. Proper Butterfat Differentials between Fruit and Vanilla Ice Cream.** CARL KOERVER, The Borden Co., Brooklyn, N. Y., AND HAROLD PRATT, Philadelphia Dairy Prod. Co., Philadelphia, Pa. Proc. 37th Ann. Conv. Intern. Assoc. Ice Cream Mfgs. 2, p. 43, Oct., 1937.

Fat differentials between fruit and vanilla ice creams for the various states are given. In the majority of instances a differential of 2 per cent in fat content is allowable. In order to comply with the standards and, at the same time, use a high percentage of fruit many companies are forced to prepare a special mix for fruit ice cream.

As high as 32 per cent fruit has been used in making certain fruit ice creams. Starting with a 12 per cent mix, the fat content of the resulting ice cream would be 8.16 per cent. The reduction in fat is nearly double the differential allowed by law in many states.

Many states also have standards for total milk solids. Two states permit no reduction of the milk solids for fruit or nut ice cream; the balance permit a reduction of 2 to 5 per cent. Using 30 per cent fruit, a reduction of 6 per cent milk solids should be permitted.

The authors favor a differential between vanilla and fruit ice creams in state standards of 4 per cent fat and 6 per cent total milk solids. They urge that individual manufacturers, as well as associations see that their interests are properly protected by advocating sound regulations. M.J.M.

**292. A Simplified Solids Tester for Ice Cream Mix.** KENNETH M. RENNER, Texas Tech. College, Lubbock, Texas. Proc. 37th Ann. Conv. Intern. Assoc. Ice Cream Mfgs. 2, p. 52, Oct., 1937.

A method is described and the necessary equipment is listed for a simplified solids tester for ice cream mix. The cost of the equipment, exclusive of a Torsion Balance Moisture Scale, is approximately 12 dollars. Results given indicate that the method checks within 0.25 per cent of the Mojonnier test in every instance where comparisons were made. When used with plain condensed skim milk, results similar to those with ice cream mix were secured, but with evaporated milk the results averaged 0.15 per cent higher than the Mojonnier method. M.J.M.

**293. Consumer Preference: (a) A Study of Ice Cream Types.** P. H. TRACY, Univ. of Illinois, Urbana, Ill. Proc. 37th Ann. Conv. Intern. Assoc. Ice Cream Mfgs. 2, p. 60, Oct., 1937.

The following conclusions were drawn from a large number of consumer-tasting tests of ice cream:

1. Body and texture are very important factors in influencing consumer preference for ice cream. Consumers preferred an ice cream having a smooth body and texture.

2. The flavor of a medium fat ice cream was preferred to that of a high fat content.

3. The high serum solids ice cream was preferred to the medium serum solids ice cream.

4. Approximately 25 per cent of the testers preferred a homemade type of ice cream to the regular commercial type of ice cream.

5. From the standpoint of this test a stabilizer is a desirable constituent of ice cream.

6. Ice cream containing a medium yellow color was preferred to uncolored ice cream.

7. The preference for vanillas varied greatly.

8. The medium and high sugar content ice cream was preferred to a low sugar content ice cream.

9. A greater percentage of women than men showed a preference for ice cream with a pronounced flavor and a heavy body. M.J.M.

**294. Consumer Preference: (b) Effect of Serving Temperature.** W. H. E. REID, Univ. of Missouri, Columbia, Mo. Proc. 37th Ann. Conv. Intern. Assoc. Ice Cream Mfgs. 2, p. 72, Oct., 1937.

The results of this investigation indicate that the serving temperature is a factor of considerable importance in determining consumer acceptance of ice cream and sherbets. Dipping properties and the stability of the products studied are also affected by the serving temperature.

The most desirable temperature for serving most ice cream and sherbets was 10° F. However products with mild flavors or a low sugar content were preferred at a higher temperature than 10° F. and those with strong flavors or high sugar content were more acceptable at temperatures below 10° F. The flavor became more pronounced in all ice creams and sherbets as the temperature was increased from 6° F. to 18° F.

The body of both ice cream and sherbets was termed too resistant at 6° F. Sherbets were criticized at 14° F. or higher as being soggy and lacking in resistance. The same criticism was made of ice creams served at 18° F.

M.J.M.

**295. Sanitary Factors at the Fountain other than Ice Cream.** F. W. FABIAN, Michigan State College, East Lansing, Mich. Proc. 37th Ann. Conv. Intern. Assoc. Ice Cream Mfgs. 2, p. 90, Oct., 1937.

A survey was made of twenty-one drug stores and twenty-nine restaurants and dairy bars in an attempt to study from a bacteriological viewpoint each item entering into the serving of ice cream.

The work indicates that there should be a bacterial standard for dipper water and that a satisfactory standard would be the same as the maximum permissible bacterial count allowed for ice cream.

Of the flavors and syrups tested, chocolate syrup usually was the heaviest contaminated and contributed the most bacteria to a dish of ice cream. Strawberry, cherry, pineapple, peach, butterscotch, lemon and orange syrups were sources of contamination, they are listed in the order of the average bacterial content. A bacterial standard of 5,000 bacteria per cc. is proposed for the flavoring and fruit syrups used with ice cream.

Unless proper precautions are observed, ice cream can be grossly contaminated during serving. Dipper water contamination usually is greater

than that from chocolate and fruit syrups. Spoon and dish contamination are negligible if they are washed in soapy wash water at 110 to 120° F. containing less than 50,000 bacteria per cc., then rinsed in water at 170° F. for at least a minute or for a minute or longer in clean water containing 50 to 200 p.p.m. of available chlorine.

Forty-six per cent of the ice cream sold in Lansing and East Lansing, Michigan, was found to exceed the legal bacterial standards of 150,000 bacteria per cc. M.J.M.

**296. A Cooperative Quality Improvement Program.** E. H. PARFITT, Purdue Univ., Lafayette, Ind. Proc. 37th Ann. Conv. Intern. Assoc. Ice Cream Mfgs. 2, p. 100, Oct., 1937.

The quality improvement program sponsored by Purdue University for Indiana ice cream manufacturers was presented. The results of the analysis and scoring of ice cream samples was given in tabular form. The success of the project is indicated by the increased number of commercial samples submitted each year. M.J.M.

**297. Best Selling Flavors.** Ice Cream Trade J. 34, 1, p. 9, Jan., 1938.

Information collected by Esmund, Gundlach and Co., Cincinnati, Ohio, from large and small manufacturers located in all parts of the United States and Canada, indicated that the 12 most popular flavors of ice cream were vanilla, strawberry, chocolate, cherry, maple nut, orange pineapple, butter pecan, black walnut, banana, peach, lemon, and pineapple. Next to the above came chocolate variations, fruit salad, almond toffee, butterscotch, peppermint stick, tutti fruttii, caramel, and pecan krunch. Sherbets were rated as follows: orange, pineapple, raspberry, lemon, and lime. W.H.M.

**298. The Year's Research Record.** J. C. HENING, New York Agr. Exp. Sta., Geneva, N. Y. Ice Cream Trade J. 34, 1, p. 24, Jan., 1938.

Subjects discussed briefly are: homogenization by sonic vibration, a new vacuum method of freezing fresh milk and cream for use in ice cream, problems encountered in making ice cream of a high butterfat content, the use of cerelese in ice cream, comparison of different serum solids concentrates for ice cream, sodium alginate as a stabilizer for ices, *E. coli* as an index of pasteurization efficiency, sanitary procedure for handling ingredients added to the mix after pasteurization. W.H.M.

**299. How to Figure Mix Costs.** E. L. REICHART, The Univ. of Nebraska, Lincoln, Nebr. Ice Cream Trade J. 34, 2, p. 22, Feb., 1938.

The cost of ice cream mix varies greatly depending upon the cost and availability of the various ingredients used and upon the composition of the mix. Generally a plant with an output of less than 20,000 gallons annually is better off to buy mix. In territories where fresh products are available,

mixes can be best and cheapest made using milk, cream and condensed skim as sources of fat and serum solids. In localities removed from actual production areas, mixes are cheapest when made from butter, skimmilk powder or other concentrates.  
W.H.M.

300. **Sales Increase 300 Per Cent.** LUCIOUS S. FLINT. Ice Cream Trade J. 34, 2, p. 12, Feb., 1938.

Details are given for a sales campaign used by Edy's Grand Ice Cream Company, Oakland, California, which has materially increased the amount of ice cream sold to their dealers. The plan is built around a monthly feature special and the use of point-of-sale display material. Carefully worked out receipts are furnished the dealers and a great deal of personal contact work is done to assure the success of the campaign.  
W.H.M.

Other abstracts of interest are numbers 267, 271, and 303.

## MILK

301. **Bitterness and Thinning in Canned Cream.** A. A. NICHOLS, G. R. HOWAT AND C. J. JACKSON. J. Dairy Research 8, p. 346, 1937.

Three organisms, all strains of *Bacillus subtilis*, were isolated from defective commercially-canned cream which, on inoculation into normal cream, caused bitterness and thinning. Some thirty-six strains of *B. subtilis* were isolated from canned dairy products but only a few were capable of producing the defects when inoculated into normal cream. The spores of these organisms were found capable of withstanding temperatures up to 120° C. for as long as 40 minutes. The defects developed more rapidly by incubation at 37° C. than at lower temperatures. Determinations of the non-protein nitrogen and of the peptone and sub-peptone fractions indicated that the development of both bitterness and thinning were related to the breakdown of the protein. Owing to the high thermal death point of the organisms responsible for the defects, it was expected that control under commercial conditions must depend more on improving the quality of the incoming milk supply than on altering the conditions of sterilization of the canned product.  
S.T.C.

302. **Soft Curd Milk of the Mineral Modified Type.** Anonymous. Milk Dealer 27, 6, p. 43, March, 1938.

A brief description of soft-curd milk of the Mineral modified type and its sale in Chicago.  
C.J.B.

303. **The Problem of Recontamination of Pasteurized Milk and Its Products.** L. C. BULMER, Bureau of Food and Dairy Inspection, Jefferson Co. Bd. of Health, Birmingham, Ala. Milk Dealer 27, 6, p. 76, March, 1938.

Based on a study of conditions which prevail in all the 51 major cities of America, with population over 200,000, this paper deals not only with the vexed problem of the promiscuous handling of pasteurized milk and ice-cream mix, but also the relationship between boards of health and industry at the present time.

The author also points out that Birmingham is the only city in America that has regulations which adequately control this problem. C.J.B.

**304. Sweet Acidophilus Milk.** Anonymous. *Milk Dealer* 27, 6, p. 116, March, 1938.

A description of how sweet acidophilus milk can be made in your own plant with no added equipment and at a marked reduction in cost to the consumer. C.J.B.

**305. Uniformity of Cream Line.** JOSEPH BURNS, Capitol Dairy, Madison, Wis. *Milk Dealer* 27, 6, p. 120, March, 1938.

A brief discussion of the importance from a sales standpoint of having a uniform cream line.

The author suggests observation of the following points in order to maintain a uniform cream line.

1. Have definite routine production.
  2. Never heat milk above 145° (excepting in flash system).
  3. Avoid use of live steam in pasteurizing jackets as much as possible.
  4. Have sufficient refrigerating capacity so that milk need not be cooled by water in pasteurizer jacket prior to sending over surface cooler.
  5. Avoid excess agitation during holding period and in storage tanks.
  6. Avoid holding periods over 30 minutes.
  7. Have thermometer in water jacket of pasteurizer so that if heating by hot water, the temperature of the heating medium can be available (usually about 160° to 175°).
- C.J.B.

Other abstracts of interest are numbers 267, 268, 269, 270, and 271.

## PHYSIOLOGY

**306. The Immature Rat Uterus as an Assay Endpoint for Gonadotropic Substances.** C. G. HELLER, HENRY LAUSON AND E. L. SEVRINGHAUS. Dept. of Medicine, Univ. of Wisconsin, Madison. *Amer. J. Physiol.* 121, 2, p. 364, Feb., 1938.

Two hundred and twenty-eight immature female rats were used. The minimal dose producing uterine enlargement (as judged by gross inspection) and uterine weight increases were only one-eighth as large as the minimal dose which resulted in ovary weight increase. The curves rise rapidly to a maximum and very slowly recede, so that only a small portion, at the lowest dose levels, is useful for assay purposes. These workers found that the ovary actually decreased in weight at first as the result of gonado-

tropic stimulation while with larger doses it responds both by increased secretion and growth. An addendum to the paper states that on recalculation of the data of Levin and Tyndale a ratio of 3 or 4 to 1 is obtained, as contrasted to their ratio of 8 to 1. D.L.E.

**307. Changes in the Water of Tissues Induced by Diets Containing Various Mineral Supplements.** E. S. EPPRIGHT AND A. H. SMITH, Lab. of Physiological Chemistry, Yale Univ. School of Medicine, New Haven. *Amer. J. Physiol.* 121, 2, p. 379, Feb., 1938.

The electrolyte balance of the diet influences the hydration of the tissues. When sodium chloride constitutes the only mineral supplement to the salt-poor basal diet of rats, the tissues, except skeletal muscles, are more hydrated than normal. Potassium exerts a slightly modifying effect. When the mineral supplement consists mainly of calcium, the general tendency is toward dehydration.

The normal distribution of water in the organism depends to a considerable extent upon the balance of the calcium with its related elements and the alkali metals sodium and potassium. In the absence of calcium and phosphorus, muscle tissue contains more sodium than can be accounted for by that in the extracellular water as calculated from the chloride present. D.L.E.

**308. The Nature of Magnesium Tetany.** D. M. GREENBERG AND E. V. TUFTS, Division of Biochemistry, Univ. of California Medical School, Berkeley. *Amer. J. Physiol.* 121, 2, p. 416, Feb., 1938.

A study has been made on the influence of a number of factors on the incidence, time of onset, and duration of peripheral vasodilation and hyperirritability in magnesium deficient rats. These symptoms were found to be greatly affected by the degree of magnesium deficiency, the starting age of the rats, and the dietary levels of calcium and vitamin G. At levels of less than 1 mgm. of Mg per 100 grams of food all experimental animals reacted within 10 to 14 days and their total life span was from 21 to 30 days with death resulting from a spontaneous convulsion. Probably the explanation of the apparent synergism of the two deficiencies lies in the fact that vitamin G deficiency itself promoted some damage to the nervous system which would tend to increase its reactivity so that the effects of the two deficiencies are additive. The hissing of an air blast appeared to be peculiarly effective in producing convulsions in hyperirritable individuals.

The localization of the lesion concerned with magnesium hyperirritability appears to be in the midbrain or pons as contrasted with thyroid or low calcium tetany, with which, lesions are more likely located in the neuromuscular junction. At least magnesium tetany differs from calcium tetany in that curare does not prevent the onset of convulsive seizures in this condition.

A later paper of this series (*ibid.*, p. 424) describes the effect of renal insufficiency upon the kidneys. D.L.E.

**309. The Influence of Calcium and Potassium upon Intestinal Absorption.** J. W. GARDNER AND G. E. BURGET, Dept. of Physiology, Univ. of Oregon Medical School. *Amer. J. Physiol.* 121, 2, p. 475, Feb., 1938.

Potassium chloride added to a 10 per cent glucose solution in concentrations from 0.03 per cent to 0.15 per cent increases the rate of absorption of the sugar solution from chronic closed intestinal loops in dogs; in similar concentrations  $\text{CaCl}_2$  decreases the rate of absorption below normal. The increase produced by a 0.1 per cent solution of KCl is approximately equal to the decrease brought about by a similar concentration of  $\text{CaCl}_2$ . The favorable action of KCl reaches a maximum at about 0.08 per cent under these conditions. The retarding action of  $\text{CaCl}_2$  increases steadily up to 0.15 per cent which was the highest concentration used. Similar results were obtained with rats by a slightly different technique. The authors state "if these results may be explained by alteration of cell permeability by the two electrolytes, our work conforms to the well established effects of K and Ca ions on cell permeability." D.L.E.

**310. Enterocrinin, a Hormone Which Excites the Glands of the Small Intestine.** E. S. NASSET, Dept. of Vital Economics, Univ. of Rochester, Rochester, New York. *Amer. J. Physiol.* 121, 2, p. 481, Feb., 1938.

Enterocrinin is the name given to an intestinal hormone, not previously described, which plays an important rôle in the secretion of succus entericus. This hormone is obtainable from the small and large intestines of several species of animals. The small gut of the dog and the cow have the highest titre of the animals examined.

The secretagogue activity of crude extracts of these organs is not directly related to blood pressure changes because vasodilation-free enterocrinin has been prepared. Enterocrinin does not excite the pancreas; hence, it is separate and distinct from secretin. It augments the secretion of enzymes as well as fluid, a property usually not ascribed to secretin. D.L.E.

**311. The Effects of Hypophysectomy and of Anterior Pituitary Extracts in the Disposition of Fed Carbohydrates in Rats.** JANE A. RUSSELL, Institute of Experimental Biology, Univ. of California, Berkeley. *Amer. J. Physiol.* 121, 3, p. 755, March, 1938.

In the hypophysectomized rats, the proportion of absorbed glucose oxidized and the proportion of total calories obtained from carbohydrate were both much higher than in the normal rats. Decreases in the amount of

glycogen stored were accounted for by the increases in the rate of oxidation of carbohydrate. It was concluded that the anterior pituitary is concerned not only with preservation of body carbohydrate during fasting, but also with the disposition of this substance when it is fed. D.L.E.

**312. Further Studies of Intestinal Absorption with the Performance of Osmotic Work.** RAYMOND C. INGRAHAM AND MAURICE B. VISSCHER, Dept. of Physiology, Univ. of Illinois, Chicago, and Univ. of Minnesota, Minneapolis. *Amer. J. Physiol.* 121, 3, p. 771, March, 1938.

The authors present a unique theory to explain the different rates of absorption of univalent and polyvalent ions from the intestine. In the presence of poly-univalent salts ( $MgCl_2$ ,  $CaCl_2$  and others) and poly-polyvalent salts ( $MgSO_4$ ,  $CaSO_4$  and others) uni-univalent salts (such as  $NaCl$ ) are rapidly absorbed from the intestine against blood plasma concentration gradients as much as 28 to 1 and 10 to 1 respectively. A slow absorption of the polyvalent ions occurs at the same time. While univalent anion impoverishment is occurring in the intestine the pH of the gut fluid invariably becomes more acid, around pH 6.1 to 6.3, while with univalent cation impoverishment the gut fluid becomes more alkaline, reaching pH 7.9.

Without exception ammonia production increases in the gut during this univalent impoverishment. The concentration of  $NH_3-N$  in the gut fluid increases to as much as two hundred and fifty times its concentration in the blood. The fact that intravenous injection of ammonium salts produces a very large increase in the  $NH_3-N$  content of the gut indicates that the ammonia in the intestinal fluid is of metabolic and not of bacterial origin.

The authors suggest that the selective transport of materials against high concentration gradients is a result of the circulation of fluid through differentially permeable membranes. They believe that there is a continuous flow of fluid into and out of the gut during absorption. D.L.E.

**313. The Blood Volume of Normal Dogs.** JOHN H. GIBSON, 2ND, JOHN L. KEELEY AND MICHEL PIJOAN, Lab. of Surgical Research and Dept. of Medicine, Harvard Medical School. *Amer. J. Physiol.* 121, 3, p. 800, March, 1938.

The merits of the various techniques used in determining blood volume are discussed. Original data on plasma, cell and total blood volume, hematocrit and hemoglobin value of venous blood, and blood velocity rate of 50 dogs are presented. In terms of cubic centimeters per kilogram in dogs of from 5 to 30 kgm. in weight, plasma volume ranged from 41.2 cc. to 51.7 cc.; cell volume, from 36.4 cc. to 54.6 cc.; and total blood volume, from 84.0 cc. to 97.3 cc. No distinct difference in plasma, cell or total blood volume in relation to weight exists between male and female dogs. With increase in body

weight, and the accompanying increase in total blood volume, there is an increase in hematocrit and hemoglobin values, and a slowing of blood velocity rate. This indicates that total blood volume bears a direct relationship chiefly to the amount of muscular tissue in the animal. D.L.E.

**314. Further Evidence for a Mammogenic Hormone in the Anterior Pituitary.** E. T. GOMEZ AND C. W. TURNER, Dept. of Dairy Husbandry, Missouri Agr. Exp. Sta. Proc. Soc. Exp. Biol. Med. 37, 4, p. 607, Jan., 1938.

Further evidence is presented indicating that the pituitary gland is the seat of production of a factor or factors which stimulate the growth of the duct and lobule-alveolar systems of the mammary gland. This principle, called the "mammogenic hormone," is present in the pituitaries of cattle when pregnant. The growth of the mammary glands of castrated female rabbits and rats was stimulated by the daily injection of such pituitaries. That this principle is not identical with the lactogenic hormone is indicated by the lack of response with non-pregnant cattle pituitaries containing considerable amounts of the lactogenic hormone. S.W.M.



# JOURNAL OF DAIRY SCIENCE

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## ABSTRACTS OF LITERATURE

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International Association of Milk Dealers	The Royal Technical College, Copenhagen, Denmark
National Institute for Research in Dairying, Reading, England	United States Department of Agriculture
New York Association of Dairy and Milk Inspectors	

## ABSTRACTS OF LITERATURE

### BACTERIOLOGY

- 315. Standard Agar Counts as Compared with Counts on Improved Agars at 32° C.** M. W. YALE, N. Y. Agr. Exp. Sta., Geneva, N. Y. *Am. J. Pub. Health* 28, 2, p. 148, 1938.

Data collected by 56 laboratories showing comparative agar plate counts of 23,715 samples of dairy products are summarized. The advantages to be gained through the use of an improved agar and an improved incubation temperature are discussed. M.W.Y.

- 316. Disintegration of Paper Board for Bacteriological Examination.** J. R. SANBORN, N. Y. Agr. Exp. Sta., Geneva, N. Y. *Am. J. Pub. Health* 28, 5, p. 576, 1938.

The technic used for the disintegration of paper board for determination of total bacterial counts is described. The apparatus consists of an electrically operated food mixer with double propellers which exert a beating action on the fibers. The paper is reconverted to a uniform pulp suspension suitable for distribution in ordinary Petri plates. The technic has been used in connection with studies of paper milk containers. M.W.Y.

- 317. Suitable Paper Wrappers and Containers for Foods.** J. R. SANBORN, N. Y. Agr. Exp. Sta., Geneva, N. Y. *Am. J. Pub. Health* 28, 5, p. 571, 1938.

The sanitary control of paper milk containers and paper containers for foods in general is discussed. Topics included are (1) fundamental requirements for the production of paper of satisfactory sanitary quality; (2) types of microorganisms in container board; (3) bacterial counts of container board; (4) sanitary standards for container board; (5) handling of clean wrappers and container board and (6) examination of fabricated paper containers for milk. M.W.Y.

- 318. Microbial Flora of Paper Containers.** FRED W. TANNER, Laboratories of Bact. of Univ. of Ill., and American Can Co., Maywood, Ill. *Am. J. Pub. Health* 28, 5, p. 587, 1938.

The author concludes that the average bacterial content of paper milk containers for distribution of fluid milk is much lower than the counts which have been reported for some glass bottles. The types of bacteria are usually *sarcinae*, white staphylococci, aerobic sporeforming rods, and non-spore forming rods. Such organisms are of no sanitary significance according to the author. M.W.Y.

319. **Contribution to the Study of Lactic Bacteriotherapie. Lactic Ferments and Fermented Milks.** ALEXANDER NEUKOMM, Nestle and Anglo-Swiss Condensed Milk Co., Vevey, Switzerland. *Le Lait* 18, 174, p. 353, April, 1938.

The various organisms present in the cultures used in preparing such milks as Yoghourt, Koumiss, and others are described and the possible mechanism of lactic acid formation is discussed. The chemical composition of these milks is also reported. Forty literature citations are given.

A.H.J.

## BREEDING

320. **A Statistical Inquiry into the Inheritance of Milk Yield in Three Herds of Dairy Shorthorn Cattle.** A. D. BUCHANAN SMITH. *Jour. of Dairy Research* 8, 347-388. 1937.

The paper embodies an examination of the possibility of sex-linked genes affecting the transmission of milk yield. To reduce environmental effect the study was restricted to three herds of the English Dairy Shorthorn breed. Fisher's squared difference method was used. The lumped results indicate that the paternal grandsire has a lesser effect than the maternal grandsire. In this respect the figures for one herd did not agree with the other two. The subject is reviewed, and the author concludes that the accuracy of the conclusions which may be drawn from statistical studies of this type are not commensurate with the labor involved.

S.T.C.

## BUTTER

321. **Use of Anti-oxidants to Prevent Tallowiness in Butter.** W. J. CORBETT AND P. H. TRACY, Univ. of Ill., Urbana, Ill. *Nat. Butter and Cheese J.* 28, 24, p. 10, Dec. 25, 1937.

The addition of 1 per cent Avenex (finely powdered oat flour) on a fat basis retards the development of oxidized flavors in butter. The addition of an aqueous filtrate of Avenex appears to be the most practical procedure of adding it to the cream. Avenex should be added to the cream after neutralization and before pasteurization. The addition of Avenol (an extract of oat flour with an oil solvent hexane) gave less positive results than did the Avenex. Wrapping prints in Avenized parchment (made by sizing parchment paper in oat flour solution) retards the development of surface flavors and improves slightly the keeping quality of the butter.

W.V.P.

322. **What Needs to be Done to Increase the Consumption of Butter?** L. C. THOMSEN, Univ. of Wisconsin, Madison, Wis. *Nat. Butter and Cheese J.* 29, 2, p. 10, Jan. 25, 1938.

There is an apparent drop in butter consumption probably because people regard butter as fattening and expensive. Consumption may decrease still more with the declining proportion of children in the population and the increased advertising of butter substitutes. Butter advertising should stress that educators and doctors favor the use of butter. The adult population must be honestly convinced that butter is not expensive considering calorie and vitamin content and that when it is used intelligently it is not fattening. Improving the condition of milk production, methods of manufacture, flavor and color control should make advertising campaigns more effective. W.V.P.

**323. Making No. 1 Butter from No. 2 Cream.** L. P. SHARPLES, Milk Processes Inc., Philadelphia, Pa. *Nat. Butter and Cheese J.* 29, 6, p. 7, Mar. 25, 1938.

"Plastic cream" is chilled cream with 80 to 83 per cent of butterfat. Compared to fluid cream it is cheaper to ship and store. It is made by passing heated fluid cream through a special centrifugal separator which removes curd, dirt (if the cream is neutralized), and nine-tenths of skim milk. The bowl of the centrifuge is automatically self-cleaning. The cream is pasteurized after coming from the centrifuge and is passed over a special stainless steel drum cooler. There is no extra cost for making plastic cream when cream for storage is produced from whole milk. When fluid cream is received instead of whole milk the extra cost is estimated as one-eighth cent per pound. Plastic cream is used for making fluid cream, ice cream, cream cheese, flavored spreads and butter. W.V.P.

**324. Technological Exploration of the Art of Buttermaking.** M. E. PARKER, American Butter Inst., Chicago, Ill. *Food Research* 3, 1 and 2, p. 261, Jan.-Feb., Mar.-Apr., 1938.

An interesting discussion is given of the keeping qualities of butter and some problems of manufacture. F.J.D.

**325. The Determination of the Quantity of Diacetyl in Butter.** R. DEHOVE AND L. DESSIRIER, Municipal Lab. of Lille, France. *Le Lait* 18, 172, p. 150, Feb., 1938.

The usual methods of determining diacetyl based on the formation of dimethylglyoxime or of quinoxaline, or of xyloquinone were tested on 50 gram samples of butter but were not found sensitive enough for determining the diacetyl in this small quantity of butter. The dimethylglyoxime method was accordingly modified. Twenty cc. of distillate obtained from the 50 grams of butter were treated with 1 cc. of 10 per cent chlorhydrate of hydroxyamine followed by 2 cc. of normal soda solution. After agitation 1 cc.

of a 0.2 per cent solution of nickel sulphate was added and then 0.6 cc. of normal acetic acid. The whole solution was then transferred to a porcelain dish and evaporated on a water bath. The residue was taken up 3 times with 2 cc. of chloroform, the filtrate and washings were evaporated to dryness on a small white porcelain dish. The nickel dimethylglyoximate then deposited as a reddish violet coloration on the white porcelain. The quantity of diacetyl in the sample was then determined by comparison with the colors in test dishes prepared in the same manner from quantities of diacetyl varying from 0.05 milligram to 0.50 milligram corresponding to diacetyl contents of the butter varying from 1 to 10 milligrams per kilogram. Another method of determining diacetyl was also investigated depending on the intense color produced in sulphuric acid medium by pyrocatechol and resorcin in the presence of traces of diacetyl. A.H.J.

Other abstracts of interest are numbers 316, 317, 318, 344, 345, 351, 368, 369, and 371.

## CHEESE

326. **Buttermilk and Cottage Cheese.** C. R. NICKOLLS, H. P. Hood and Sons, Inc., Boston, Mass. Ann. Conv. Intern. Assoc. Milk Dealers, Plant Section, p. 11, 1937.

For cultured buttermilk pasteurization of skimmilk at 180°F. or more for 20 to 30 minutes is recommended. A system of carrying starters and three methods of producing butter granules are proposed. Little agitation and storage temperatures below 50°F. are emphasized.

In order to control texture and flavor of cottage cheese more accurately extracts or coagulators are recommended and the setting period is confined to 12 hours. The curd is cut just before wheying off occurs at near .8 per cent acidity. Stirring only every 15 or 20 minutes and other details in order to produce best results are specified. E.F.G.

327. **Natural Cheese with Package Appeal.** ANONYMOUS. Nat. Butter and Cheese J. 29, 1, p. 14, Jan. 10, 1937.

The mechanical method of making and packaging a soft-ripened type of cheese is described. Pasteurized-milk curd is formed into long slabs of uniform size and weight. These are cut into small loaves or bricks after curing. The treatment permits ripening with minimum rind development; reduces the cost of manufacture; and makes the cheese more attractive to the distributor and consumer. Patents for the special equipment have been granted to Raymond Miollis, Natural Cheese Inc., Chicago, Illinois.

W.V.P.

- 328. A Quality Improvement Program Proposed for the Wisconsin Cheese Industry.** W. V. PRICE, Univ. of Wisconsin, Madison. Nat. Butter and Cheese J. 29, 6, p. 18, Mar. 25, 1938.

A plan is suggested to secure compensation for the voluntary production of an approved or "certified" type of American cheese. Standards of production, manufacture and handling would be defined by an elected "Council" of industry members representing farmers, manufacturers and dealers. The cooperation of interested public health agencies would be invited in formulating these definitions. The Council would determine suitable compensation to producers for meeting these standards and would control the necessary inspection, branding and analytical services which would be paid for by participating factories. Costs of these services are estimated at approximately 0.2 cents per pound of approved cheese.

W.V.P.

- 329. Canning of Cheddar Cheese.** L. A. ROGERS, Bureau Dairy Industry, U. S. Dept. of Agr., Washington, D. C. Food Research 3, 1 and 2, p. 267, Jan.-Feb., Mar.-Apr., 1938.

The development of canned cheddar cheese is discussed together with its present status and future possibilities.

F.J.D.

- 330. Utilization of Whey in Foods.** B. H. WEBB, Bureau Dairy Industry, U. S. Dept. Agr., Washington, D. C. Food Research 3, 1 and 2, p. 233, Jan.-Feb., Mar.-Apr., 1938.

The author discusses the work of the B. D. I. in attempting to find uses for cheese factory whey. Some success has been experienced in utilizing whey in certain cream soups, tomato juice, fruit mixtures and drinks and fruit whips. Sweetened condensed whey appears to offer interesting possibilities.

F.J.D.

- 331. The Identification of Roquefort Cheese.** G. GENIN, Paris, France. Le Lait 18, 174, p. 372, April, 1938.

Genuine Roquefort cheese is made entirely from sheep's milk. Sheep's milk fat is characterized by a considerably higher caprylic and capric acid content. On the analysis of the fat this appears as a higher Polenske number. The higher Polenske number of the fat from cheese of the Roquefort type consequently indicates whether it has been made from cow's or from sheep's milk. Cheese fat having a Polenske number of 3 or lower indicates the cheese was not made from sheep's milk. The fat used in determining the Polenske number may be extracted with ether or allowed to drain from a warmed sample that has been well worked to soften it. The feed given the cows or sheep does not cause the difference in Polenske numbers of fats

from the two milks as even when cows and sheep receive the same feed there is the characteristic difference in Polenske numbers of the fats from the two milks.  
A.H.J.

Other abstracts of interest are numbers 351, 368, and 369.

## CHEMISTRY

- 332. Action of Enzymes at Low Temperatures.** A. K. BELLS AND HANS LINEWEAVER, U. S. Dept. of Agr., Washington, D. C. Food Research 3, 1 and 2, p. 57, Jan.-Feb., Mar.-Apr., 1938.

The authors conclude that enzyme action at low temperatures not only takes place but is an important factor in food preservation. The velocity of reaction in some cases, especially with lipase, is surprising. In others it is so slight as to be detected with uncertainty. However, even in the latter cases the initial phase of the reaction may have been completed so that when the food is brought to ordinary temperatures again the spoilage occurs with greater rapidity.  
F.J.D.

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

- 333. Advancement in Sterilization Methods for Canned Foods.** C. O. BALL, American Can Co., Maywood, Ill. Food Research 3, 1 and 2, p. 13, Jan.-Feb., Mar.-Apr., 1938.

The author presents a very complete historical review of the subject with bibliography, and list of patents, some of which deals with evaporated milk or with methods which might be applicable to evaporated milk, particularly "high-short sterilization" methods.  
F.J.D.

- 334. Distinctive Characteristics of Skimmilk Powders\*(Powders Soluble—Powders by the Hatmaker Process).** JEAN PIEN, Lab. of the Farmers Union, Paris, France. Le Lait 18, 174, 347, April, 1938.

Finely ground skimmilk powders prepared by the Hatmaker process have the same appearance as the more soluble powders made by the spray process. They may be distinguished, however, by their appearance under the microscope. Skimmilk powder, made by the Hatmaker process, consists of irregularly shaped plates, while spray powder is made up of spheres. When the two types of powder have been mixed, microscopic examination serves to detect such mixture. Spray powders are considerably more soluble than powders made by the Hatmaker process. When 10 grams of the latter powder is mixed with 100 cc. of water and allowed to stand, the precipitate will occupy 50 to 90 cc. Spray process powders similarly tested will show a precipitate of only 1 cc. or less. Skimmilk powders made by the Hatmaker

process are lower in bacterial count than spray skimmilk powders. The reduction time for methylene blue is accordingly much longer for the Hatmaker type of skimmilk powder. Reconstituted spray skimmilk powder sours after standing for a short time, while reconstituted Hatmaker powder may stand for several days without souring. A.H.J.

**335. Artificial Wool from Casein.** P. DIATCHENKO. *Le Lait* 18, 175, p. 233, March, 1938.

The properties of casein of importance for its use in the making of synthetic wool are: the moisture content because of its effect on the stability in connection with subsequent dyeing, the fat content because of its effect on the quality of the textile fibers and on filtering the casein solution before casting the threads, the ash content because of the effect on the viscosity of the casein solution, and on the quality of the fibers, and the acidity because high acidities of the casein produce transformations in the properties of casein. The process of making synthetic wool involves the following steps—preparation of a casein solution, preparation of the fiber, construction of the thread, fixation of the thread and drying and finishing of the thread. Casein is usually dissolved according to 2 procedures—by ammonium hydroxide or by caustic soda plus carbon disulphide. The solutions produced by the two methods are filtered through cotton and then pass to machines where, under pressure, they are forced through small openings into the fixing solution containing acid and other compounds including formalin. After washing and drying, the fibers prepared in this manner have the appearance and feeling of wool. The synthetic wool prepared by the ammonia method is of greater fineness than natural wool or synthetic wools prepared by the carbon disulphid method or the Italian method. The resistance to pull and the extensibility of the synthetic wool prepared by the ammonia process approach more closely these properties of natural wool than do the synthetic wools prepared by the carbon disulphide method or by Italian Lanatal. A.H.J.

Other abstracts of interest are numbers 315, 336, 337, 342, 344, 345, 351, 368, and 369.

## DISEASES

**336. The Effect of Mastitis on the Udder and Its Product.** T. S. SUTTON, Ohio State University, Columbus, Ohio. *Ann. Conv. Intern. Assoc. Milk Dealers, Prod. Section*, p. 3, 1937.

The causal organisms and changes in the udder during the progress of the disease are outlined. Changes in the milk may be classified as physical, chemical and biological. The physical changes are those of consistency and

color or appearance. Chemically, mastitis milk usually shows an increase in chlorides, catalase, albumin, and pH, and a decrease in lactose and casein. The biological changes usually noted are increases in leucocytes and bacteria, the bacteria being chiefly long chain Streptococci. E.F.G.

337. **The Composition of Milk as Affected by Mastitis.** C. H. WHITNAH, W. J. CAULFIELD, A. C. FAY, AND V. D. FOLTZ, Kansas Agr. Exp. Sta., Manhattan, Kansas. Ann. Conv. Intern. Assoc. Milk Dealers, Prod. Section, p. 19, 1937.

A numerical mastitis milk score is obtained based upon leucocyte and bacteria counts. These were compared with chemical determinations of minerals, sugar, protein, lecithin, lipase, phosphatase, carotene, flavin, and vitamin C, on 50 to 70 cows for a period of two years. The data confirm and extend to additional constituents the conclusion of other workers, that latent mastitis does not produce uniform serious changes in the composition of milk. E.F.G.

338. **A Study of the Vaginal Content of Pregnant Bang-Infected Cows for the Presence of *Brucella abortus*.** C. P. FITCH, W. L. BOYD, AND LUCILLE M. BISHOP, University of Minnesota, St. Paul, Minnesota. J. Am. Vet. Med. Assoc., N. S. 45, 2, p. 171, Feb., 1938.

Examinations of swabs taken from the vagina of 58 reacting animals, indicate that *Brucella abortus* is not ordinarily found in the vagina of pregnant, Bang-infected cows until very shortly before an abortion or a normal parturition.

However, the organism appears in the discharges after the seal of pregnancy is broken. The investigators cautioned that the animals examined did not show noticeable discharges from the vagina and that animals showing such discharges might be exceptions. J.W.W.

339. **Bang's Disease—Status of Vaccination.** W. WISNICKY, Wis. Dept. of Agr. and Markets, Madison, Wis. Ann. Conv. Intern. Assoc. Milk Dealers, Prod. Section, p. 72, 1937.

About 20 per cent of abortions in cattle are produced by causes other than infection with the Bang organism. In recent years it has been proved that the abortion bacterins are practically worthless in the control and treatment of Bang's disease. The virulent live culture vaccines do have some merit. However, they possess disadvantages which more than outweigh any good that they may have. In the use of the virulent live culture vaccine, the disease was actually introduced into herds that were free from the disease. More recent researches indicate that by the use of a moderately virulent strain of the Bang organism on young calves, a degree of immunity against the disease may be established. E.F.G.

340. **Bang's Disease in Cattle.** A. E. WIGHT AND J. M. BUCK, U. S. Dept. of Agr., Beltsville, Md. Ann. Conv. Intern. Assoc. Milk Dealers, p. 60, 1937.

Since July, 1934, when Federal funds were made available for conducting supervisory work in the field and making payments to owners whose cattle reacted to the test, steady progress has been made until at present 15.3 per cent of the cows of the United States are under supervision. During the fiscal year ended June 30, 1937, agglutination tests were applied to 8,000,000 cattle with 398,000 or 5 per cent reacting. The history of attempts to control the disease with vaccines and similar means is traced. Some success with calfhood vaccination under carefully controlled conditions with a vaccine from *Br. abortus* strain 19 has been attained under experimental conditions.  
E.F.G.

341. **Pathological Changes Occurring in the Bovine Udder Due to Infectious Mastitis.** W. T. MILLER, Bureau of Animal Industry, Beltsville, Md. Ann. Conv. Intern. Assoc. Milk Dealers, Prod. Section, p. 5, 1937.

This study is restricted to the effects of cocci entering the udder through the teat canal. Examination of 304 udders showed 256 or 84 per cent were carrying streptococci in one or more quarters so that the study was largely one of lesions occurring in chronic streptococcus mastitis. The normal course of this infection was an infiltration of leucocytes followed by the formation of increasing quantities of connective and scar tissue with the ultimate almost complete disappearance of normal alveoli in longstanding cases of marked mastitis. The various conditions are illustrated with ten photomicrographs.  
E.F.G.

## FOOD VALUE OF DAIRY PRODUCTS

342. **The Nutritive Value of Milk Supplemented with Minerals as an Exclusive Diet for Rats. Comparison of Equal Volumes of Summer and "Winter" Milk Before and After Laboratory Pasteurization.** K. M. HENRY, E. W. IKIN, AND S. K. KON. J. Dairy Research 8, p. 282, 1937.

Milk was obtained simultaneously from cows on early pasture and from stall-fed cows receiving winter rations. By suitable blending of the morning's and evening's milkings of each milk the fat content of the two milks was equalized daily. A part of each milk was then pasteurized in the laboratory by a "holder" method. The total nutritive value of the milk was measured on rats in two separate experiments. In one the milks, supplemented with iron, copper and manganese, were fed as an exclusive diet.

The four types of milk were given to twelve groups of litter-mate male rats, the intake being equalized within each group. The experiment lasted 8 weeks, and at the end no difference was found in gain in weight, body length, general appearance of the rats or composition of the carcasses. The palatability of the milks as gauged by the refusals of the rats were investigated by various statistical methods, which showed that summer milk was probably more palatable than "winter" milk, but that pasteurization had no effect.

In the second experiment the intake of milk was limited to 20 ml. daily, but the rats were given in addition unlimited access to a basal diet of casein, sugar and salts. This experiment was also carried out on groups of four litter mates (four groups of does and seven groups of bucks). After 8 weeks 5 per cent brewer's yeast was added to the basal diet, resulting in a marked increase of the growth rate of all the groups. At the end of 8 weeks, the gains in weight, intake of basal diet, and gains per gram of solids ingested were compared. The only statistically significant differences were in favor of pasteurized summer milk when compared with summer raw and with "winter" pasteurized.

Taking both experiments into consideration it is concluded that they did not reveal any difference in the total nutritive value of the milks. S.T.C.

343. **Carotene and Ascorbic Acid Content of Fresh Market and Commercially Frozen Fruits and Vegetables.** G. A. FITZGERALD, Birds Eye Labs., Boston, Mass., AND C. R. FELLERS, Mass. Agr. Exp. Sta., Amherst, Mass. *Food Research* 3, 1 and 2, p. 109, Jan.-Feb., Mar.-Apr., 1938.

Data showing that the vitamin content varies considerably between different fruits and vegetables and between the same ones grown or packed in different places. Vitamin A is almost totally retained during processing and freezing and also during storage. F.J.D.

344. **The Nutritive and Safety Value of Pasteurized Milk.** GORDON BATES, Health League of Canada, Toronto, Canada. *Ann. Conv. Intern. Assoc. Milk Dealers*, General Sessions, p. 11, 1937.

Experience of Toronto from health standpoint, where compulsory pasteurization has been in effect since 1915, is cited to indicate the effectiveness of pasteurization since not a single case of bovine tuberculosis infection has been encountered in this generation of children raised on pasteurized milk in Toronto. Also, among 100,000 children admitted to the hospital for sick children there has not been a single case of abdominal tuberculosis since inauguration of pasteurization. The progress of pasteurization in Canada and United States is discussed.

Evidence is given to indicate that the nutritive properties of milk are

not injured by pasteurization, and the author recommends that all milk be pasteurized. E.F.G.

345. **The Status of Vitamin A as Related to Dairy Cattle.** O. C. COPELAND, Texas Agr. Exp. Sta., College Station, Texas. Ann. Conv. Intern. Assoc. Milk Dealers, Prod. Section, p. 28, 1937.

Effects of vitamin A deficiency in feed upon vitamin A content of resulting butter are shown. Cows fed on cottonseed meal and hulls produced fat with  $2\frac{1}{2}$  units vitamin A potency per gram. With the above, plus sorghum silage, 12 per gram, whereas with pasture also added the butterfat contained 33 units per gram. On a vitamin A deficient diet, the most rapid decrease in potency of butterfat occurs during the first 60 days. Two cows fed a vitamin A deficient ration decreased from 40 units to 12 units per gram, but on 5 hours pasture daily came back to original 40 units at end of third day. It seems that green pasture is necessary for the production of butterfat which can be classed as high in vitamin A potency. Cows in such an advanced stage of vitamin A deficiency that they cannot gain their feet, recover rapidly when vitamin A is administered. E.F.G.

## HERD MANAGEMENT

346. **Die Bestimmung der Milchleistungsfähigkeit.** (Measuring milk producing ability. Part 2.) L. KRÜGER. Züchtungskunde 13(2), 49-63. February, 1938.

The average discrepancy between a cow's uncorrected record and her real producing ability is about 20 per cent of the record in data from cow testing associations in Silesia. In about one-fourth of the cases the discrepancy exceeds 40 per cent. Various methods of making these discrepancies smaller so that they will be less serious obstacles to successful selections are compared. It is not very practical to avoid all corrections by (1) absolute standardization of conditions beforehand, or (2) by providing physiologically optimum conditions for one or more "test" lactations, or (3) by selecting afterward those lactations made under conditions which happened to be "standard." Those procedures are sometimes helpful but often reduce the errors only a little and can rarely include more than about a quarter of all records, even when the range of conditions included as "standard" is made wider than is desirable. The use of correction factors (as for age, service period, lactation length, etc.) helps but is never complete and does not adjust for general conditions of feeding or management to which all cows of the herd are exposed. The method recommended is to correct the lactations to the production at a standard age during a calving interval of definite length (360 days) and with a dry period and preceding dry period of definite length, by means of suitable correction factors. Then this standard-

ized record is corrected for general conditions of feeding and management by comparing it with the production of other cows freshening near the same time in the same herd. It is claimed that the average discrepancy between a record corrected by Krüger's method and the cow's real ability is only about 9 per cent.

J.L.L.

## ICE CREAM

347. "Maple—Favorite Food Flavor." FREDERICK S. MORISON. *Food Ind.* 10, 3, p. 143, 1938.

This article is a discussion of the grades and uses of maple syrup. A concentrated syrup about two and one-half times the strength of ordinary maple syrup is now available which yields maximum flavor with minimum amount of sweetening.

E.O.A.

348. Sound Waves—A New Tool for Food Manufacturers. L. A. CHAMBERS. *Food Ind.* 10, 3, p. 133, 1938.

A general discussion is presented of the theories regarding dispersion of particles by the sonic vibrator and the uses of the equipment in the milk and ice cream plant.

E.O.A.

349. Sodium Alginate as a Stabilizer. MERRILL J. MACK. *Food Ind.* 10, 4, p. 195, 1938.

This article is a digest of the practical application of sodium alginate as a stabilizer in ice cream, market cream, sour cream, sweet cream, cheese spreads, chocolate milk and ices and sherbets.

E.O.A.

Other abstracts of interest are numbers 315, 316, 317, 318, 321, 332, 334, 342, 343, 344, 345, 351, 355, 356, 359, 364, 368, 369, 370, and 371.

## MILK

350. Why Confusion in the Milk Industry? F. J. BAHL, Mathews Frechtling Dairy Co., Cincinnati, Ohio. *Milk Dealer* 27, 7, p. 40, 90, April, 1938.

The author points out that the fresh milk industry can no longer ignore canned milk as its competition. Canned, evaporated milk is here to stay, and the idea of pegging fluid milk prices according to the whim and fancies of any particular group is no longer possible.

The production of fluid milk does not vary importantly from the production of milk for evaporating purposes; and the idea of pegging fluid milk market continually and having its competitor, evaporated milk, buy on a formula basis is not logical. If it is logical, possible and practical to buy

milk for evaporating on a formula basis, a ratio can be established for fresh milk. It is time that this matter is presented to the government and other interested parties in a decisive way. No man can afford to ignore his competition any longer—either in the buying end or in the sales end.

A definite fixed ratio of one cent a quart more to a producer for producing fresh milk is suggested. C.J.B.

**351. Cause of Black Spots in Milk Cans.** (ANONYMOUS.) Milk Dealer 27, 7, p. 42, April, 1938.

Report of an investigation showing that black spots in milk cans are frequently caused by leaving a stirrer of pure nickel in the can of milk.

The explanation given is that the black spots were caused by electrolytic action set up by the contact of two different metals; the lactic acid in the milk, slight as it was, setting up a condition similar to that found in a wet battery, the resulting electrolysis causing oxidation of the tin in the form of black spots, all with that characteristic jagged, uneven formation.

C.J.B.

**352. Individual Tumblers of Milk for Restaurant Trade.** (ANONYMOUS.) Milk Dealer 27, 7, p. 43, April, 1938.

A description is given of how the Harding Restaurants in Chicago are serving pasteurized, homogenized milk in individual tumblers which the waiters open before the customers.

C.J.B.

**353. Improving the Quality of Milk.** A. A. BORLAND, Penn. State College, State College, Pa. Milk Dealer 27, p. 62, April, 1938.

A discussion on improving the quality of milk in which the author deals with flavor, cooling milk at the farm, cleaning utensils, color and vitamins in milk, inspection at the intake, and paying a bonus for premium milk.

C.J.B.

**354. Daylight vs. Night Delivery.** F. E. ROGERS, Thompson's Dairy, Washington, D. C. Milk Dealer 27, 7, p. 56, April, 1938.

A discussion of the advantages, disadvantages, and consumer reaction to daylight delivery. The author sums the pros and cons of the subject by saying that daylight delivery has the preponderance of theoretical advantages. Practically, however, there are some very potent disadvantages to a widespread adoption of daylight service universally.

C.J.B.

**355. The Phosphatase Test as a Means of Determining Efficiency of Milk Pasteurization.** HOMER L. SPENCER, City Health Dept., Tulsa. Milk Dealer 27, 6, p. 66, March, 1938.

A description of the principles upon which the phosphatase test is based. Modifications of the original test are also given. The author concludes that it can be stated that although this test is probably not perfect in its present form, and that it may be modified from time to time as experience dictates and as needs for new application arise, the phosphatase test as devised by Kay and Graham offers the most accurate and sensitive means for determining the efficiency of pasteurization at present available. C.J.B.

**356. Present Status of Phosphatase Test for Pasteurization.** WALTER VON DOHLEN TIEDEMAN, State Dept. of Health, Albany, N. Y. *Am. J. Pub. Health* 28, 3, p. 316, 1938.

The author believes that the phosphatase test for pasteurization has great practical value and reviews typical examples of violations that were found by the use of this test. Its great value to the milk control official is discussed by Paul F. Krueger, Director, Bureau of Dairy Products, Board of Health, Chicago, Illinois, and by Sol Pincus, Deputy Commissioner, Department of Health, New York, N. Y. M.W.Y.

**357. Application of Scientific Control in the Bottling Industry.** MAX LEVINE, Dept. of Bact., Iowa State College, Ames, Iowa. *Food Research* 3, 1 and 2, p. 141, Jan.-Feb., Mar.-Apr., 1938.

The problems of the bottling industry are discussed. The concentrations of sodium hydroxide in bottle washing with relation to thermal period to destroy bacteria are given. These data are applicable in the washing of milk bottles.

° F.	Per cent NaOH				
	1.0	1.5	2.0	2.5	3.0
	Time to kill (minutes) *				
110	432.0	209.0	125.0	83.8	60.4
120	210.0	102.0	60.8	40.7	29.4
130	103.0	49.5	29.6	19.8	14.3
140	49.8	24.1	14.4	9.7	7.0
150	24.2	11.7	7.0	4.7	3.4
160	11.8	5.7	3.4	2.3	1.6
170	5.7	2.8	1.7	1.1	0.8
180	2.8	1.3	0.8	0.5	0.4

F.J.D.

**358. Some Recent Advances in Dairy Technology.** J. A. TOBEY, American Inst. of Baking, New York. *Food Research* 3, 1 and 2, p. 211, Jan.-Feb., Mar.-Apr., 1938.

The author discusses in a general non-technical manner the advances in

the dairy industry over the last 100 years, stressing particularly pasteurization, knowledge of nutrition, and irradiation.  
F.J.D.

- 359. Researches on the Pasteurization of Milks for Human Consumption. I. Choice of a Culture Medium for the Counting of Microorganisms.** G. GUITTONNEAU, G. MOCQUOT, AND A. EYRARD, National Lab. of the Dairy Industry. *Le Lait* 18, 173, p. 226, March, 1938.

It is stated as a general principle that a medium to be used in determining the bacterial content of milk should contain milk. Because of the opacity of media containing milk, the milk is subjected to a tryptic digestion for 3 days at 37°C (98.6°F.). Skimmilk treated in such a manner and diluted to 3 times its original volume constitutes a satisfactory media. The results obtained with pasteurized and raw milks were equivalent to those obtained when media containing small amounts of milk or lactose plus other sugars and peptones, *i.e.*, the media proposed by Bowers and Hucker and by Demeter.  
A.H.J.

- 360. Address of the President.** R. C. FISHER, Intern. Assoc. Milk Dealers, Wellesley Farms Dairy, Wellesley Farms, Mass. Ann. Conv. Intern. Assoc. Milk Dealers, General Sessions, p. 11, 1937.

Progress of the organization and the milk industry during the past year is reviewed. Accomplishments of the following active committees are listed: Sanitary procedure, transportation, laboratory methods, simplified practice, accident prevention, public relations, and legislative. The service known as "The Balanced Job of Selling and Route Supervision" is explained. The aggressive policy of the Association with reference to public relations promoted under the auspices of the Milk Industry Foundation, is designed to pave the way for more effective sales promotion by the membership. National Milk Week has made a distinct contribution and offers possibilities for greater usefulness.  
E.F.G.

- 361. Buying Plans. Ratio Between Cream Fat and Whole Milk Prices.** M. J. METZGER, Bowman Dairy Co., Chicago, Illinois. Ann. Conv. Intern. Assoc. Milk Dealers, Prod. Section, p. 34, 1937.

The use of the method by which a premium over the evaporated milk formula price based upon the butter and cheese market now used in Chicago, is discussed.  
E.F.G.

- 362. Uniformity of Dairy Score Card and Inspection.** WALTER D. TIEDEMAN, N. Y. State Dept. of Health, Albany, N. Y. Ann. Conv. Intern. Assoc. Milk Dealers, Prod. Section, p. 40, 1937.

Overlapping milk sheds have created a situation where some dairy farms are under the inspection of as many as 9 inspectors and 8 different sets of regulations. Progress in elimination of conflicting regulations in New York State is reported; also some uniformity with New Jersey requirements. Further progress in uniformity must wait upon some interstate or government action. E.F.G.

**363. Production Trends.** JOHN B. SHEPARD, Sr. Agr. Statistician, U. S. Dept. of Agr., Washington, D. C. Ann. Conv. Intern. Assoc. Milk Dealers, Prod. Section, p. 47, 1937.

Over a 56 year period it appears that consumption has increased from around 750 pounds milk equivalent per person to 825 pounds or only about 10 per cent. Three things must be kept in mind. First, milk cows are beef and may be sold as beef. Second, milk cows are machines and may be slowed down or speeded up by price of feed. Third, many farmers will milk less when times are good and their time is worth more or more congenial labor is available. The fifteen year cattle cycle reached a low point in 1935. Possibly a larger proportion of the milk will, in the future, come from herds where machine methods can be used. E.F.G.

**364. Plate Type Heat Exchangers.** G. F. POPPENSIEK, Borden's Farm Prod., New York City. Ann. Conv. Intern. Assoc. Milk Dealers, Plant Section, p. 63, 1937.

The development of plate heat exchangers used in Europe since 1923 and introduced into the U. S. in 1927 is traced. The writer reports the method of installation, operation and results from 32 of these heaters replacing various internal and external cooling units and heaters of various sorts. Favorable results are reported due to high regenerative efficiency, ease of cleaning, economy of floor space, etc. E.F.G.

**365. Necessary Changes in the Average Plant to Comply with U. S. Public Health Service Standards Ordinance and Code.** PAUL F. KRUEGER, Board of Health, Chicago, Illinois. Ann. Conv. Intern. Assoc. Milk Dealers, Plant Section, p. 67, 1937.

A detailed discussion of the requirements of the ordinance with respect to buildings and floors, walls and ceilings, ventilation pipes and fittings, cleaning equipments and bottles and pasteurization. E.F.G.

**366. Reports of Simplified Practice Committee.** A. H. LUEDICKE, Ann. Conv. Intern. Assoc. Milk Dealers, Plant Section, p. 75, 1937.

Two new solderless fittings are announced. The American Society for Testing Materials has organized Committee C-14 on Glass and Glass Prod-

ucts and glass bottle problems are being presented to this committee. A change in standards for glass milk bottles and a reduction of tolerances on stainless tubing are being proposed. E.F.G.

**367. Milk Machinery of the Future.** JOHN FORSLEW, Bowman Dairy Co., Chicago, Illinois. Ann. Conv. Intern. Assoc. Milk Dealers, Plant Section, p. 3, 1937.

The present trends in pasteurizing equipment suggest the possible more extensive use of short time high temperature pasteurization and eventually means other than heat for the destruction of bacteria. Bottle washers might combine the advantages of hydraulic and soaker types and incorporate better bottle cooling and automatic bottle feed. Bottle fillers, milk containers, fittings, can washers, can dumpers, milk cans, can washers and refrigeration are discussed from standpoint of probable future developments. E.F.G.

Other abstracts of interest are numbers 315, 316, 317, 318, 319, 326, 332, 336, 337, 338, 339, 340, 341, 342, 344, 345, 368, 369, 370, and 371.

## MISCELLANEOUS

**368. What's New in Farm Science.** Part I. 54th Ann. Report of Director, year ending June 30, 1937. Agr. Exp. Sta., Univ. of Wisconsin, Madison, Wis., Bull. 439, Dec., 1937.

This bulletin briefly summarizes findings of work newly completed or in progress. The following sections are of interest to the dairy industry:

"Grass juice factor" is a new vitamin. G. O. Kohler, C. A. Elvehjem, and E. B. Hart, p. 2.

Progress in study of vitamin B. C. A. Elvehjem, A. Arnold, and E. B. Hart, p. 4.

A new dietary factor in the vitamin B complex. D. V. Frost, C. A. Elvehjem, p. 5.

Pigs and calves need fat along with skimmilk. E. J. Schautz, C. A. Elvehjem, and E. B. Hart, p. 10.

Nitrogen compounds in livestock feeding. E. B. Hart, H. J. Deobald, and G. Bohstedt, p. 11.

Tests on A. I. V. silage and milk. W. H. Peterson, G. Bohstedt, E. B. Hart, *et al.*, p. 14.

Who operates Wisconsin milk plants? A. W. Colebank, R. K. Frokker, and A. C. Hoffman, p. 35.

Accuracy of tuberculin testing. E. G. Hastings and J. R. McCarter, p. 53.

Spoilage of cheese brines. G. B. Landerkin and W. C. Frazier, p. 60.

Studies on Swiss cheese starter cultures. H. J. Pepler, P. R. Elliker, and W. C. Frazier, p. 61.

Vitamin C, oxidized flavor and the test for copper in milk. J. P. Turgeon, V. C. Stebnitz, and H. H. Sommer, p. 63.

Studies on lipase activity. K. G. Weckel, H. C. Jackson, and D. W. Jones, p. 65.

Microscopic examination of cheese. H. Templeton, D. K. Stewart, and H. H. Sommer, p. 66.

Test for extraneous matter in cheese. D. W. Spicer and W. V. Price, p. 67.

Acidity control in Brick cheese. D. W. Spicer and W. V. Price, p. 68.

Design of milk irradiating equipment. H. H. Beck, H. C. Jackson and K. G. Weckel, p. 70.

Abnormal milk and mastitis. E. G. Hastings, p. 75.

Bang's disease. M. R. Irwin, L. C. Ferguson, B. A. Beach, and G. C. Humphrey, p. 77. W.V.P.

**369. The Use of Metals in the Dairy Industry. I. Aluminum.** G. GENIN, Paris, France. *Le Lait* 18, 172, p. 113, Feb., 1938.

A review is given of the properties of aluminum as a material for the fabrication of dairy equipment and the behavior of aluminum in contact with dairy products. Among the phases of the subject discussed are: the historical development of metallic aluminum, aluminum alloys, methods of fabricating aluminum, commercial forms of aluminum, physical, chemical and mechanical properties of aluminum, superficial treatment of aluminum to allow painting, burnishing and polishing, the workability of aluminum, the corrosion of aluminum by milk, sterilizing solutions, brine and alkaline cleaning solutions. It is noted that many alkaline solutions are very corrosive to aluminum, but a solution containing 0.5 to 5.0 per cent sodium carbonate and 0.5 to 1.0 per cent of silicate of sodium has little corrosive effect. A.H.J.

**370. The Cash Value of Safety.** A. A. NICHOLSON, The Texas Co., New York City. *Ann. Conv. Intern. Assoc. Milk Dealers, General Sessions*, p. 34, 1937.

Accident costs are divisible two ways, the first a tangible cost, the second an intangible cost. This discussion concerns the former. Compensation insurance is based upon 60 per cent representing the loss and 40 per cent the expense. The 40 per cent breaks down as 17.5 per cent acquisition of new business, 2.5 per cent to taxes, 2 per cent to inspection and safety, 2.5 per cent to payroll audits, 8 per cent claim expense and 7.5 per cent home office expense. Manner of arriving at both "manual rate" and "countrywide rates" is explained. The "manual rate" can be reduced by practical and successful safety programs and results in savings to the industry.

Intangible costs have been rated on the basis of 4 to 1. That is, for each dollar paid in direct compensation, medical attention, etc., through personal injury to an employee, the organization loses an additional four dollars. Management has no greater opportunity of expressing the human

side of itself and its employees than through a safety program. People like to work for an organization that stands for a sound, Christian-like fundamental such as safety.

E.F.G.

## PHYSIOLOGY

- 371. Concerning the Production of Alkaline Milk and of an Increase of the Iodine Index in the Fatty Matter in Milk Following Subcutaneous Injection of Dinitrophenol.** E. BROUWER AND J. MARTIN, Exp. Sta., Dept. of Phys., Hoorn, Holland. *Le Lait* 18, 174, p. 337, April, 1938.

After the subcutaneous injection of dinitrophenol in the goat, there was observed a production of alkaline milk (pH 7.67–7.75), and the carbon dioxide content also showed a marked increase (up to 98 volume per cent). In the butterfat, there was also an increase in the iodine index of about 15 units. The diminution of the feed furnished the animals analogously caused an increase in the iodine index because under such conditions the reserve fat is utilized for the production of fat in the milk. The pH and acidity of the milk from the goats on the reduced ration however remained normal. It is probable that when the dinitrophenol is injected, it is absence of foodstuffs (because of feverish conditions of the goats and diminished appetite) that causes the change in iodine index. After each injection of a significant quantity, the milk assumes a yellow color due to the presence in the milk serum of dinitrophenol either as such or modified. The significance of the results are discussed from the standpoint of the physiology of the goat.

A.H.J.



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National Institute for Research in Dairying, Reading, England	United States Department of Agriculture
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## ABSTRACTS OF LITERATURE

### BACTERIOLOGY

- 372. How Sterilizing Agents Act on Bacteria.** I. L. BALDWIN, Univ. of Wisconsin, Madison, Wisconsin. Ann. Conv. Intern. Assoc. Milk Dealers, Laboratory Section, p. 88, 1937.

Among micro-organisms the power to reproduce is commonly accepted as the single criterion of life or death. Death of micro-organisms is usually caused by some interference with one or more of the three basic chemical equilibria on which life depends. These are the oxidation reduction equilibrium from which the energy for life processes is derived; the hydrolytic-polymerization equilibrium which is responsible for the building up and tearing apart of certain complex constituents of the cell; and the acid-base equilibrium which serves as a regulator responsible for the building up and tearing apart of certain complex constituents of the cell.

It seems probable that the lethal action of increased acidity or alkalinity upon bacteria is largely due to an increase in the rate of hydrolysis with consequent destruction of protoplasmic constituents. The action of chlorine and hypochlorites is due partially to oxidizing action and partially to their tendency to unite with protoplasmic constituents of the bacterial cell. Destructive forces are the removal of water by drying or the addition of sugar or salt. The lethal action of heat is more effective if moist or if the medium is acid or alkaline and the effect seems to be due to a combination of hydrolysis and coagulation.

E.F.G.

- 373. Spoilage of Cream at Low Temperatures.** J. A. ANDERSON, Department of Bacteriology, Rutgers University. Ann. Conv. Intern. Assoc. Milk Dealers, Laboratory Section, p. 19, 1937.

A new bacterium for which the name *Bacterium lipidis* is proposed exhibits powerful fat splitting action, weak action on protein, and none on sugars growing rapidly at refrigeration temperatures. A sharp throat irritation results from swallowing cream containing the organism and this is thought to be due to liberation of caproic, iso-caproic, and caprylic acids from the milk fat. Spoilage of cream at low temperature is due mainly to hydrolysis of fat and protein which accounts for the rancid and bitter flavors.

E.F.G.

- 374. A Further Report on Tabulations of Counts Using Proposed Changes in Medium and Temperature of Incubation of Milk Samples.** ERNEST KELLY, Chief, Division of Market Milk Investigations, Bureau of Dairy Industry, U. S. D. A., Washington, D. C. Ann. Conv. Intern. Assoc. Milk Dealers, Laboratory Section, p. 25, 1937.

Tables show that standard agar at 32° F. gives more uniform results between plants than tryptone agar at either 37° C. or 32° C. Grade A pasteurized milk seemed to be one of the few exceptions to this. There was greater variation in the results from different laboratories on the same sample of milk with the same medium than there was between the results in the same laboratory using different media and temperatures. It would seem that laboratory procedure needs to be standardized and that greater care to control conditions be exercised if satisfactory results are to be obtained.

E.F.G.

**375. Dairy Bacteriology.** BERNARD W. HAMMER. 2nd Ed., 1938, Wiley, 482 pp., price \$5.00.

Dr. Hammer has brought this book up to date by including in the second edition the main facts which have been brought out by research workers during the 10 years since the first edition was published. The book is broad in scope as evidenced by the following list of headings of the 15 chapters:

Bacterial counts of milk; milk fermentations; contamination of milk and cream and its control; growth of organisms in milk and cream; body cells in milk; spread of diseases through milk and its derivatives; preservation of milk and cream; milk enzymes; bacteriology of evaporated, sweetened condensed and dry milk; bacteriology of ice cream; bacteriology of butter cultures; bacteriology of fermented milk preparations; bacteriology of butter; bacteriology of cheese; tests for the general quality of milk and cream.

Dr. Hammer's broad experience in practically all lines of dairy bacteriology and his ability to write clearly on difficult subjects has resulted in a book which is outstanding.

M.W.Y.

**376. Bacterium in Milk.** HERMANN OESER, Pr. Forsch. F. Milchwirtschaft in Kiel. Zentr. Bact. 2, 96, p. 287, 1937.

The problem was to reveal which were the principal representatives of the *Bacterium coli aerogenes* group in raw market milk. The enrichments from the different media were plated on Endofuchsin agar and analyzed primarily after the technique used in German medical laboratories. Furthermore, the behavior in litmus milk, the gas formation in skimmilk at

	Indol	Meth. Red	V. - P.	Citr.	Nr. of strains
1	+	+	-	-	56
2	+	+	-	+	7
3	-	+	-	-	26
4	-	+	-	+	18
5	+	+	+	-	1
6	+	+	+	+	26
7	-	+	+	+	22
8	-	-	+	+	8

30° C., the ability to use citric acid as a sole source of carbon have been determined. The so-called reaction combinations with the known four differential tests gave results as shown in the accompanying table: Some of the combinations found by Ruchhoft *et al.* and Demeter and Sauer could not be detected. There was a remarkable constancy of the Indol and the citrate test and a more or less noticeable variability of the M. R. and V.-P. test. The gas test in skimmilk proved to be a valuable means for differentiation: typical *B. coli* strains produce only a few cc., typical *B. aerogenes* and very many intermediates, however, relatively high amounts of gas. All tests made have been summarized and there were found 25 combinations of theoretically 128 possibilities with 164 strains. Finally the author tried to put the 164 strains into the system of Bergey with the result that he got 12 subgroups. He did not encounter the 7 *Escherichia* species and 4 *Aerobacter* species of Bergey. *E. Aerogenes* does not belong to the E. A. group at all and the species *E. acidi lactici* and *E. anindolica* are to be discarded.

K.J.D.

## BUTTER

**377. How to Determine the Keeping Quality of Butter.** C. H. PARSONS, Swift and Company, Chicago, Illinois. Nat. Butter and Cheese J. 29, 7, p. 6, April 10, 1938.

Creameries need a simple test to indicate the quality which butter will have after it has passed through the regular trade channels. A test was developed which has been use for practically five years on thousands of samples of butter. This test consists of holding wrapped, printed butter which has been judged for quality for fourteen days at a temperature of 60° F. At the end of this period of time this butter is again judged for quality. The difference in score indicates the deterioration which the butter has undergone during the test. The butter is always tempered at 45 to 50° F. before judging. Careful control of temperatures of incubation is necessary. Incubation temperatures of 68 to 70° F. for a period of eight days gives approximately the same result as holding for fourteen days at 60° F. Oiling-off of the product may occur at the higher temperatures. If this occurs the test is practically valueless.

W.V.P.

**378. How Should Cream be Held at the Creamery?** C. C. TOTMAN, S. Dakota State College, Brookings, S. Dak. Nat. Butter and Cheese J. 29, 8, p. 34, April 25, 1938.

Cream of No. 1 grade or better was divided into three lots and subjected to different treatments. Lot 1 was neutralized to .25 per cent acidity and pasteurized at 145° F. for 30 minutes, cooled and held in the cream vat for 4 to 15 hours before churning. Holding temperatures were 47 to 56° F. Lot No. 2 received essentially the same treatment except that it was stored

in cans for 40 to 44 hours at temperatures varying between 35 to 45° F. Lot No. 3 was held in cans for 40 to 44 hours at temperatures of 35 to 45° F. and was then neutralized and pasteurized in the manner described. Cream from Lot 1 produced butter of lower quality. The decline in scores of butter from this lot was quite noticeable during the last three months of storage. There seemed to be a slight advantage in holding cream raw under the conditions of these experiments. It is suggested that the effects of neutralizer, metallic salts and heat may be responsible for changes in the flavor of the butterfat in cream held for long periods; or that low acidity in neutralized and pasteurized cream during the holding period might favor the development of proteolytic and lipolytic bacteria. W.V.P.

Other abstracts of interest are numbers 373, 432, 447 and 459.

## BUTTERMILK

379. **How to Make Flake Buttermilk by Spraying Fat into Cultured Skim Milk.** E. H. PARFITT. *Milk Plant Mo.* 27, 3, p. 27, March, 1938.

The author presents the details of the Vogt method of making flake buttermilk by spraying liquid fat into cultured buttermilk. A comparative body and flavor study of (a) churned-cream buttermilk, (b) churned-flake buttermilk, and (c) Vogt-method buttermilk showed that the body of the churned-cream buttermilk was free from lumps and poured like sweet milk. The other two buttermilks possessed rather heavy bodies. The churned-cream buttermilk flavor was preferred by those accustomed to the "old-fashioned" buttermilk but the flavors of all three types were maintained splendidly for five days. G.M.T.

## CHEESE

380. **A Study of Inexpensive Pasteurizing Units for Cheese Factories.** WALTER V. PRICE and LEO GERMAINE. Univ. of Wisconsin, Madison, Wis. *Nat. Butter and Cheese J.* 29, 7, p. 14, April 10, 1938.

Two relatively inexpensive types of pasteurizing equipment which are suitable for use in cheese factories receiving less than 10,000 pounds of milk per day are described. The bacterial destruction efficiency of these two types of equipment were tested by the plate count method. Scores are shown for cheese made from raw milk and from identical milk pasteurized by one or the other of these two types of equipment. Both methods of pasteurizing were sufficiently effective bacteriologically to improve the quality of the cheese. W.V.P.

381. **Influence of Manufacturing Methods on Acidity of Brick Cheese.** S. W. SPICER and WALTER V. PRICE, Univ. of Wisconsin, Madison, Wis. *Nat. Butter and Cheese J.* 29, 10, p. 18, May 25, 1938.

When brick cheese was made from pasteurized milk inoculated with commercial type *S. lactis* starter it was found that the amount of starter, the ripening period before adding the rennet, the temperature of heating, and the time of dipping the curd must be carefully controlled to produce sweet cheese of desirable quality. A development of .02 per cent titratable acidity in the whey before dipping when dipping occurred  $2\frac{1}{2}$  hours after setting seemed to provide a correct rate of acid development for the remainder of the process. Heating temperatures of  $104^{\circ}$  F. in conjunction with this acid development produced a pH at three days after making of approximately 5.1 with a moisture content of 38 per cent at the time of paraffining. Cheese which satisfied these characteristics was sweet and of desirable quality. W.V.P.

**382. The Manufacture of Blue-veined Cheese in the Midwest.** C. B. LANE, Iowa Agric. Exper. Sta., Ames, Iowa. Nat. Butter and Cheese J. 29, 12, p. 14, June 25, 1938.

Steps in the curd-making process are described. The ripening of the cheese by mold and the use of homogenization for the milk for making blue cheese are discussed briefly. The development of commercial manufacture of the blue cheese in the United States is reviewed and the prediction is made that the demand for this domestic product should increase materially during the next few years if high quality standards are maintained.

W.V.P.

**383. Rate of Ripening in Cheddar Cheese.** THEODORE R. FREEMAN and C. D. DAHLE, Pennsylvania Agric. Exper. Sta., State College, Pennsylvania. Technical Bulletin 362, May, 1938.

From an economic standpoint the ripening process is one of the most important phases of cheddar cheese production. This is especially true in obtaining the desired flavor in the cheese at the time it reaches the consumer. Also, by reducing the time required to ripen cheese, the cost may be reduced.

A study was made of seventeen lots of milk which were divided into moisture, acid, Rennin, Pepsin and Trypsin Series. Each lot from the different series was divided into two equal portions and the cheese made in separate vats. One cheese from each vat was ripened at approximately  $45^{\circ}$  F. and the other at approximately  $63^{\circ}$  F.

Analysis included bacterial content and type, pH, moisture content, amino nitrogen content and flavor score, except that the nine lots in the enzyme series were not subjected to the bacterial analysis.

It is concluded that:

The rate of proteolysis in cheddar cheese during ripening is directly related to the numbers of bacteria initially found in the cheese, as deter-

mined by the total count made on lactose and skimmilk agars and by the proteolytic count made on skimmilk agar.

There is no relationship between numbers of bacteria initially found in cheese and the development of flavor in the same cheese.

Changing the moisture content of the cheese through slight modifications in the curd-making process cannot be expected to influence materially the rate of ripening in the cheese.

The rate of proteolysis in cheddar cheese ripening can be increased 40 to 100 per cent by raising the ripening temperature from 45° to 63° F.

The maximum flavor score is reached more quickly when the cheese is ripened at 63° F. than when it is ripened at 45° F.

Cheese ripened at 63° F. will attain as high a maximum flavor score as that ripened at 45° F.

Low initial acidity in the cheese is conducive to more rapid proteolysis but has no significant effect on the rate of flavor development.

The quality of the aged cheese, as judged by flavor score, is slightly inferior in the low-acid cheese.

The occurrence of bitter flavor during aging is favored by a high ripening temperature.

Bitter flavor in cheese appears to be due to the presence of one or more of the substances resulting from the breaking down of casein.

Additional amounts of pure rennin increase the rate of proteolysis in cheddar cheese ripening, increase slightly the rate at which the flavor develops, and produce an aged product with slightly higher flavor score.

Added pepsin increases the rate of proteolysis during cheddar cheese ripening particularly at the beginning of the ripening period and at the lower ripening temperature, does not accelerate the development of flavor, but produces an aged product with appreciably higher flavor score.

Trypsin increases markedly the rate of proteolysis during the early part of the ripening period, after which its effect is greatly reduced. This enzyme also increases slightly the rate at which flavor develops, but reduced the maximum flavor score attained.

W.D.S.

Other abstracts of interest are numbers 432 and 459.

## CHEMISTRY

384. **The Effect of Excess of Vitamins A, B and C on the Assay of Vitamin D and of Excess Vitamin D on the Assay of Vitamin A.**  
HILDA M. BRICE and GEORGINA E. PHILLIPS, Pharmacological Laboratory of the College of the Pharmaceutical Society, London. *Biochem. J.* 32: 1-4, 1938.

The response by the line test technique for vitamin D was not influenced by giving, at the same time, excessive doses of vitamins A, B or C (40 γ

carotene, 10–15 I.U. B, and 0.15 mg. ascorbic acid, respectively, daily per test animal. The weight response to a small dose of vitamin A was not influenced by giving a graded series of excessive doses of vitamin D (8–100 I.U. per animal per week). It is concluded by the authors that when assaying vitamin A or vitamin D, it is unnecessary to consider the possible presence of another vitamin in the substance under test, provided the basal diet is adequate.

K.G.W.

**385. Buffer Intensities of Milk and Milk Constituents. III. Buffer Action of Calcium Citrate.** E. O. WHITTIER, Bureau of Dairy Industry, U. S. Dept. of Agric., Washington, D. C. *J. Biol. Chem.* 123: 283, 1938.

“Equations describing the buffer action of calcium citrate have been derived and curves based on these equations compared with curves constructed from potentiometric titrations of calcium citrate solutions. Support is given the Hastings-McLeon idea of the mechanism of ionization of calcium citrate in solution.”

“Application of the results of milk equilibria indicates that the buffer action of citrates in milk is exerted principally in the range in which phosphates and casein buffer most intensely and is of slight moment compared with the effects of these other buffer substances.”

K.G.W.

**386. Note on the Quantity of Theobromine in the Milk of Cows Fed on a Diet Including this Alkaloid.** H. C. DOWDEN, National Institute for Research in Dairying, Univ. of Reading. *Biochem. J.* 32: 71, 1938.

In 1934 it was reported that cocoa shell is rich in vitamin D, and in 1935 that the feeding of 2 pounds of shell daily for a month during the winter raised the vitamin D content of the milk of stall-fed cattle to the normal summer level, while in 1937 it was found that the fat content of these milks was increased during the period of shell feeding.

Cocoa shell contains approximately 3 per cent of theobromine, and a cow receiving 2 pounds daily of the shell would receive approximately 9 grams of the alkaloid. Theobromine was fed in 9 gram quantities daily to a small group of cows for 3 weeks and on the last day samples of milk were analyzed for theobromine content. It was observed that in the milk of three cows the average daily milk yield of which was approximately 35, 19 and 18 pounds, the “theobromine” was respectively 4.71, 2.05 and 4.72 mg. per liter. In blank tests upon the method, yields of approximately 70 per cent of added theobromine were obtained. The maximum content of theobromine transmitted to the milk of the above three cows is, therefore, approximately 7 mg. per liter. According to a supplementary publication of the British Pharmacopoeia, the medicinal dose of theobromine is 0.3–0.6

gram. For a child under 12 months, for whom the dose would be  $\frac{1}{12}$  of that prescribed for an adult, the minimum dose is contained in  $6\frac{1}{2}$  pints of the milk. Plain eating chocolate contains approximately .3 per cent theobromine, and it is not uncommon for  $\frac{1}{4}$  pound of this chocolate (containing 0.3 of theobromine) to be eaten by an adult at one time. K.G.W.

**387. Analysis of Proteins. IX. The Content in Amino Acids of the Caseinogen and Lactalbumin of Woman's Milk. R. H. A. PLIMMER and J. LOWNDES, Chem. Dept., St. Thomas's Hospital Medical School, London, S.E. 1. Biochem. J. 31: 1751, 1937.**

The protein of cow's milk and woman's milk was concurrently analyzed for amino acid content. To compare the nutritive value of the protein of cow's milk with woman's milk, the cow's milk was generally diluted with an equal volume of water.

Except for cystine, which is the same in amount in cow's and woman's milks, cow's milk contains  $\frac{2}{3}$  times the amounts of the other amino acids. Cow's milk diluted with an equal volume of water will contain half the amount of cystine present in woman's milk. If diluted with two volumes of water then tryptophane and possibly arginine will be below the amounts in woman's milk. Cow's milk diluted with an equal volume of water will have an equal amount of the sum of the sulphur bearing amino acids, cystine and methionine. K.G.W.

**388. Dialysis of Milk. III. Salt Equilibrium with Special Reference to Calcium, Magnesium, and Phosphorus. L. H. LAMPITT, J. H. BUSHILL and D. F. FILMER, Lyons Laboratories, London, W. 14. Biochem. J. 31: 1861, 1937.**

It has been previously shown by these authors that the acidity of milk is of distinct importance in determining the dialysability of certain constituents, particularly salts. The results of this study show that acidification with either diluted or concentrated acids had the same effect on the amount of dialysable constituents.

Neutralization of acidified, raw, separated milk results in an almost complete recovery of the original amounts of dialysable Ca, Mg and inorganic P, although the figure for Ca remains slightly above normal.

The effect of extended agitation (14 days) on the dialysable constituents was studied. It is suggested that the salt equilibrium of milk is normally unstable and may be shifted by agitation. Treatment of the milk in its preparation for milk powder appears to stabilize the salt equilibrium.

The following ranges of analytical figures are presented for raw, pasteurized and dried, separated, "average" milk.

	Total	Dialysable (as % of total present)
Inorganic P	0.63-0.77	33-44
Organic P	0.31-0.38	7-15
Ca	1.27-1.44	25-42
Mg	0.10-0.15	62-83

K.G.W.

- 389. The Milk Clotting Action of Papain.** A. K. BALLS and S. R. HOOVER, Bur. of Chem. and Soils, U. S. D. A., Washington, D. C. *J. Biol. Chem.* 121: 737, 1937.

The milk clotting component of papain appears to possess activity in agreement with the conception for papain proteinase. The component is activated by H<sub>2</sub>S, cysteine, phenylhydrazine, and cyanide, and has a high temperature optimum.

The time required for clotting was shown to be a straight line function of the enzyme concentration; a quantity of the enzyme, constant for any condition, is inactivated by the milk.

K.G.W.

- 390. The Position of the Unsaturated Linkage in the Hexadecenoic Acids of Certain Natural Fats.** JOHN M. SPADOLA and R. W. RIEMENSCHNEIDER, Bur. of Animal Ind., U. S. D. A., Washington, D. C. *J. Biol. Chem.* 121: 787, 1937.

The hexadecenoic acid present in goat milk fat, egg yolk glycerides, and the depot fat of the white rat is chiefly the 9-, 10-hexadecenoic acid.

K.G.W.

## DISEASE

- 391. Sterility in Cattle Symptom not Disease.** C. R. DONHAM, Ohio Agric. Exper. Sta., Wooster, Ohio. *Ohio Exper. Sta. Weekly Press Bull.* XXIII-18, July 7, 1938.

Sterility in cattle is not a specific disease, but symptom of a large number of different diseases. Appropriate treatment can be applied only after intelligent diagnosis by a competent veterinarian.

W.E.K.

- 392. Results of Calfhood Vaccination.** L. J. TOMPKINS, Sheffield Farms Company, N. Y. *Cert. Milk* 13, 141, p. 7, Jan., 1938.

This article summarizes the results up to date of a field experiment in calfhood vaccination for Bang's disease which was started in January 1934. The results of the research work done show that when calves are vaccinated between the ages of four and eight months with an appropriate vaccine, the agglutination titre disappears in a relatively short time, leaving the animal immunized to some degree.

W.S.M.

Another abstract of interest is 401.

## FOOD VALUE OF DAIRY PRODUCTS

393. **Milk the Most Perfect Food.** N. N. GODBOLE, Benares Hindu Univ., Dipawali, India, 1936. Distributed by Chemical Publishing Co., 148 Lafayette St., N. Y.; Price \$1.25.

The title of this book is not sufficiently broad to include the full text, for the author really presents the dairy industry of India with special reference to improvement of the Hindu diet through increased consumption of dairy products. For American readers this style is particularly interesting for the literature on the food value of milk has not been materially increased by researches in India while knowledge of dietary conditions in India is not too general.

The promotion of the vegetarian diet is uppermost in the thoughts of the author which is essential in India for both religious and health reasons. India has neither supervision of the slaughter and handling of meats nor refrigeration in a country where summer temperatures are usually above the temperature of the human body. Fortunately, for the nutrition of the people, milk is accepted in the vegetarian diet for life need not be destroyed to secure it. Milk is served fresh in fluid form, or as ghee, butter, and fermented drinks. The total per capita yearly milk consumption is only about 85 pounds, caused principally by the very low production of the cattle. It is estimated that India has 52,500,000 milk cows and 20,500,000 she-buffaloes, the former producing 100 pounds and the latter 1200 pounds of milk per year in excess of that required by the calves.

The material in this book is presented in a very elementary manner for general public reading. Some printing errors are obvious, and exception may be taken to some of the ideas expressed by the author. Nevertheless, the book is both interesting and informative. A.C.D.

394. **The Value of Milk Protein in Infant Feeding—Part II.** P. B. CASSIDY and H. H. PERLMAN, Pediatric Society, Philadelphia, Pa. *Cert. Milk* 12, 139, p. 5, Nov., 1937.

A discussion on the following questions:

1. Is the protein of cows' milk a cause of infantile eczema?
2. Does a high temperature have a favorable or unfavorable effect upon the protein in cows' milk?
3. What is the nutritional physiologic significance of milk protein?
4. What is the comparative economic status of milk protein as found in the various forms of milk: whole milk, evaporated milk, etc.?

W.S.M.

395. **The Cow—"Mankind's Foster Mother."** W. E. KRAUSS, Ohio Agric. Exper. Sta., Wooster, Ohio. *Cert. Milk* 12, 140, p. 7, December, 1937.

This paper discusses the relationship between the feed of the cow and the composition of the milk, emphasizing the vitamin content.

W.S.M.

- 396. Soft Curd and Homogenized Milks.** IRVIN J. WOLMAN, School of Medicine, Univ. of Pennsylvania, Philadelphia, Pennsylvania. Ann. Conv. Intern. Assoc. Milk Dealers, Laboratory Section, p. 114, 1937.

The pH of the stomach contents of a healthy infant seldom falls below 5.0. The iso-electric point of casein is 4.7 while the optimum pH for action of pepsin is 2.8. Acidity in the stomach of the infant is not favorable for action of pepsin, but in the young adult stomach a pH of between 3.0 and 1.4 is reached so active digestion by pepsin is obtained.

An artificial digestion device in which milk and milk formulae were subjected to the chemical action of synthetic gastric juice made from hydrochloric acid and pepsin is described. The production of fine soft curds by the addition of banana powder and banana pulp is described. Commercial methods of producing soft curd milk are briefly discussed. Clinical evidence shows that the milk preparations which yield soft curds are well tolerated and well utilized by infants, children and older persons, and that soft curds mean fine curds. Better control and standards are needed for these products.

E.F.G.

- 397. Lime and Phosphorus, and Their Significance to the Milk Dealer.** WALTER H. EDDY, Columbia Univ., New York City. Ann. Conv. Intern. Assoc. Milk Dealers, Laboratory Section, p. 96, 1937.

The role of calcium and phosphorus in nutrition is discussed. Dr. Sherman in recommending a quart of milk per day for growing children bases this upon the fact that the child needs about 1 gram of calcium per day for body maintenance and growth and this is contained in the quart of milk. Milk is a better source of calcium than vegetables. Utilization of calcium and phosphorus is dependent upon vitamin D. A more acid condition in the digestive tract favors solubility of calcium. The parathyroid gland has a specific effect upon calcium assimilation. An outline is given of a procedure to check satisfactory calcium and phosphorus assimilation.

E.F.G.

- 398. The Effect of Season and Feeds on the Vitamin D Content of Milk Under South Dakota Conditions.** G. C. WALLIS and T. M. OLSON, South Dakota Agric. Exper. Sta., Brookings, So. Dakota. So. Dakota Exper. Sta. Bull. No. 321, March, 1938.

Six grade Holsteins were used to study the effect of the season of the year and of the feeds consumed by dairy cows on the vitamin D content

of the milk produced. A marked seasonal effect was found. Summer milk contained 32 International Units of vitamin D per quart which was about four times the amount of vitamin D contained in the winter milk produced under the conditions of this experiment.

The vitamin D in the feed eaten by the cow also had an important influence on the amount in the milk produced. When the vitamin D intake was increased the milk became proportionately richer in this important food factor. The alfalfa hay used in this experiment contained 500 International Units of vitamin D, and the Prairie hay 250 International Units of vitamin D per pound. The hay was fed at the rate of 20 pounds per day so the cows getting alfalfa hay received 10,000 International Units of vitamin D daily, and the cows getting prairie hay received 5,000 units. This difference in intake was reflected in the higher vitamin D content of the milk produced by the cows receiving alfalfa hay. However, only a small proportion (between 1 and 2 per cent in this case) of the vitamin D in the feed consumed was recovered in the milk.

Because of the importance of milk as one of the few food sources of vitamin D, advantage should be taken of all factors which will contribute towards increasing the antirachitic value of milk. Some of these factors have been indicated in this bulletin while others will require further study.

C.C.T.

**399. What If There Were No Milk?** NINA SIMMONDS, College of Dentistry, Univ. of California. *Milk Plant Mo.* 27, 2, p. 32, Feb., 1938.

Milk is discussed chiefly from the standpoint of its calcium content, the role of which in dentition is emphasized.

G.M.T.

**400. A Note on the Vitamin D Content of Cow's Colostrum.** KATHLEEN M. HENRY and S. K. KON, National Institute for Research in Dairying, Univ. of Reading. *Biochem. J.* 31: 2199, 1937.

The vitamin D content of the colostrual fats of a Guernsey cow on pasture was 1.2 I.U. per gram for the 0 and first day *post partum*, 0.56 I.U. per gram for the second, third, and first half of the fourth days, and 0.36 I.U. per gram for the last half of the fourth and first half of the fifth days. Normal control butterfat from a cow on the same ration contained 0.41 I.U. per gram. Compared with later milk, colostrum contains relatively more vitamin A and carotene than vitamin D.

K.G.W.

**401. The Relation of Fat to the Utilization of Lactose in Milk.** E. J. SCHANTZ, C. A. ELVEHJEM and E. B. HART, Dept. of Agric., Univ. of Wisconsin, Madison, Wis. *J. Biol. Chem.* 122: 381, 1938.

Rats placed on a mineralized whole milk diet made very efficient utilization of all the milk sugar. This has also been found to be true for a pig

and a calf. When the animals were placed on a mineralized *skim* milk diet, sugar was readily detected in the urine after a few days of feeding. The sugar was identified as galactose and accounted for all of the reducing material in the urine. In the case of the rat, as high as 35 per cent of the ingested galactose was recovered in the urine. Fats such as butterfat, lard, corn oil, cocoanut oil, linseed oil, and palmitic and oleic acids, when added to mineralized skim milk at levels of 3 to 4 per cent, prevented this loss in the urine. Glycerol or butyric,  $\beta$ -hydroxybutyric, caproic, and lactic acids did not prevent the loss. On mineralized skim milk the sugar of the blood rose to about 200 mg. per cent, while on whole milk it seldom rose higher than 140 mg. per cent after feeding. K.G.W.

Other abstracts of interest are numbers 384, 386, 387, 428, 444 and 454.

## HERD MANAGEMENT

402. **Some Calf Disorders of Nutritional Origin.** C. E. KNOOP, Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Exp. Sta. Weekly Press Bull. No. XXIII-6, Apr. 14, 1938.

Many calf disorders may have nutritional origin, chiefly because of lack of certain minerals and vitamins. Lack of vitamin A and iodine are cited particularly. W.E.K.

403. **Grain, Silage, Hay Supplement Pasture.** A. E. PERKINS, Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Agr. Exp. Sta. Weekly Press Bull. XXIII-10, May 12, 1938.

It is pointed out that it is seldom economical to attempt to compensate for all the deficiencies of poor pasture by means of grain only. The use of silage, hay, or a cultivated pasture crop, such as Sudan grass, in connection with a low rate of grain feeding, is recommended. W.E.K.

404. **Pasture, Cause of Milk Slump.** C. F. MONROE, Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Exp. Sta. Weekly Press Bull. No. XXIII-16, June 23, 1938.

The marked decline in milk production often experienced after the first flush from pasture is probably due to an actual shortage of palatable grass. To prevent this drop the practice of rotating pastures is suggested. W.E.K.

## ICE CREAM

405. **The Merchandising Council in 1937.** G. V. RECTOR, Fairmont Creamery Co., Omaha, Neb. Proc. 37th Ann. Conv. Int. Assn. of Ice Cream Mfrs. 4: 7, 1937.

During the past year the activities of the Ice Cream Merchandising Institute were increased. One important phase of the work was a series of 14 two-day regional merchandising conferences, so planned that they fairly covered the country geographically.

The monthly publication of the institute, "The Spinning Wheel," was continued for the benefit of sales and advertising managers of ice cream companies. Another monthly publication, "Ice Cream Currents," is planned primarily for the retail dealer. Ice cream manufacturers can purchase copies at 4 cents per copy for distribution to their dealers. Two talking slide films have been prepared for use by ice cream merchandisers; the one is intended for showing to retail dealers, the other is of interest to consumers.

The Ice Cream Merchandising Institute also offers personal information service to manufacturers with specific ice cream merchandising problems.

M.J.M.

406. **What Have You to Sell?** GEORGE W. HENNERICH, Ice Cream Merchandising Institute, Inc., Washington, D. C. Proc. 37th Ann. Conv. Int. Assn. of Ice Cream Mfrs. 10: 4, 10, 1937.

The importance of a constructive selling program is stressed by the author. Merchandising of ice cream at the soda fountain is then discussed from the standpoint of profitable operation. The home delivery of ice cream was also found to be a profitable way of selling the product.

M.J.M.

407. **Showmanship in the Ice Cream Industry.** ZENN KAUFMAN, New York, N. Y. Proc. 37th Ann. Conv. Int. Assn. of Ice Cream Mfrs. 10: 4, 20, 1937.

The author discusses the elements which he feels are essential in successful salesmanship and gives numerous illustrations of how these elements have been successfully employed.

M.J.M.

408. **Specialties—Their Place in Industry.** NORMAN THOMAS, Joe Lowe Corp., New York, N. Y. Proc. 37th Ann. Conv. Int. Assn. of Ice Cream Mfrs. 10: 4, 35, 1937.

Although bulk ice cream is the mainstay of the ice cream industry, it is true that a greater number of flavors and specialties will increase the gallons of ice cream sold in any store. Specialties which are properly packaged, stored and handled will add to the profits of an account. The ice cream salesman is urged to determine the relative amount of profit for bulk ice cream and specialties and properly instruct the retail dealer along these lines.

The ice cream maker is urged to handle only such specialties or novelties

which offer the basis of size and price control. Oversized and underpriced novelties have been the source of a considerable amount of trouble. Legal size and price control have protected numerous markets from difficulty with specialties. M.J.M.

**409. Retail Store Trends.** O'NEAL M. JOHNSON, Statistical and Accounting Bureau, I. A. I. C. M., Washington, D. C. Proc. 37th Ann. Conv. Int. Assn. of Ice Cream Mfrs. 10: 4, 44, 1937.

The author has found that 23.9 per cent of the manufacturers sell ice cream through retail stores, but the ice cream sold in this manner represents only 11.5 per cent of the total sales. Only 15 per cent of the ice cream manufacturers operate more than one retail store.

The types of stores operated, the kind of merchandise featured, the average selling prices, and the kinds of cones sold are tabulated for the ice cream stores studied. A considerable amount of data are presented and many problems arising in retail selling are discussed. A manufacturer who is considering the sale of ice cream through retail stores should find this information very helpful. M.J.M.

**410. Retail Stores. A Discussion.** Proc. 37th Ann. Conv. Int. Assn. of Ice Cream Mfrs. 10: 4, 68, 1937.

(1) LLOYD D. WITTER, Snowwhite Creameries, Inc., San Angelo, Texas.

With the advent of new developments, the ice cream industry has been changed from time to time. The retail store is a present day trend which may mean another transition point in the industry. The retail store came into existence in the depression years as a result of intense competition and decreased gallonage of sales through the regular outlets. The place of the retail store in the industry is questioned by some and upheld by others.

Retail stores will continue to be successful so long as the public is offered: Products of quality; A variety of flavors; Attractive surroundings; Convenience and service; And, most important, values.

(2) D. H. DORMAN, Protected Milk Products, Kansas City, Missouri.

Our company has operated a few retail stores over a period of four years. Until the past year the stores were not equipped for Fountain service. The addition of the fountain stores proved very successful. The retail store also proved to be very helpful in promoting specialties.

(3) E. B. DARROW, Darrow Ice Cream Co., Albuquerque, New Mexico.

Our experience with a retail store has been over only a five months' period. Nothing but package goods is sold—brick ice cream and five-cent items in package. No attempt was made to call attention to the retail store, by advertising. Yet through the store as much brick ice cream has been sold as by the ten best dealers handling our ice cream. We have found that packaged ice cream can be sold successfully through the retail store.

(4) CARL A. STEEL, Steel De Soto Ice Cream Co., Minneapolis, Minn.

A retail store was opened by our company in a town where the company operated two ice cream routes. After a few months it was found that more ice cream was being sold through the store than through the two routes.

It is believed that operating both a retail and wholesale ice cream business on a large scale is not practical. However, by operating a few retail stores it might be possible to show the dealer how to merchandise ice cream successfully by operating his store as an ice cream store during the short busy season.

(5) G. D. TURNBOW, Protected Milk Products Co., Oakland, California.

The retail store spread is only one of several fundamentals in successful operation of this type of business. Such elements as the traffic count, location of store, construction of store, products to be sold, and many other details, must be decided on before retail store spread becomes a factor.

We have found it difficult to find properly trained personnel and managers for the ice cream stores. Volume is also essential. A successful store must handle in excess of 5,000 gallons a year.

With ice milk, a gross spread of 47 per cent should be realized. Our aim for an average spread on all products is 42.5 per cent.

The proper training of new employees is paramount. They are first trained in a manner similar to that used in the classroom. Following this they work for a period of time under a trained supervisor.

The store manager is given a bonus for obtaining sales quotas on certain products and on the entire quota for the set up for the month. In addition, a special bonus is given to the managers who maintain the required store spread for the month.

M.J.M.

**411. Sodium Alginate—A Stabilizer.** V. C. STEBNITZ and H. H. SOMMER, Dept. of Dairy Industry, Univ. of Wis., Madison, Wis. *Ice Cream Field* 32, 3, p. 48, March, 1938; 4, p. 52, April, 1938.

The authors compared the stabilizing properties of sodium alginate (Dariloid) with those of gelatin of the following Bloom strengths 150, 175, 200, 225 and 250. The mixes were prepared in accordance with commercial practice except that they were processed in much smaller batches. The ice cream was frozen using a 2½ gallon vertical brine freezer.

The authors report that the addition of 0.2 per cent "Dariloid" caused an increase of 0.08 to 0.10 in the pH of the mix whereas 0.3 per cent "Dariloid" resulted in an increase of 0.13 to 0.17 pH. These additions caused a decrease in titratable acidity of 0.10 and 0.015 per cent respectively.

In contrast with the above, the various gelatins caused no appreciable change in either the pH or titratable acidity when added to the mix.

Mixes containing gelatin were found to have the same color as the control mix without any stabilizer, while the sodium alginate mixes showed slightly more color.

Measurements of viscosity showed the sodium alginate mixes to be more viscous than gelatin mixes when freshly prepared, whereas after aging this difference did not necessarily persist. They report that agitation of the sodium alginate mix caused a considerable decrease in viscosity which was not regained by subsequent aging.

No tendency to whey off occurred with the sodium alginate or gelatin mixes.

When sodium alginate mix was cooled to 60° F., aging seemed to improve its whipping ability whereas if cooled to 40° F. immediately after homogenization, aging did not improve the whipping property. The authors state "The difference in whipping ability between gelatin and sodium alginate seems to depend to a large extent upon the condition of the freezer and the amount and strength of the gelatin used—Excessive amounts of sodium alginate seemed to have the same deterrent effect on the whipping ability as excessive amounts of gelatin."

According to the authors there was practically no difference in body and texture of ice creams made with sodium alginate and gelatin when the correct amount of each was used.

The authors claim "As a stabilizer for ice cream, sodium alginate shows all the desirable properties of the other ice cream stabilizers and in addition shows some distinct advantages." In this connection they emphasize particularly the advantage of uniform viscosity during the aging of sodium alginate mixes.

W.C.C.

**412. Viennese Ice Cream Stresses Flavor.** ANONYMOUS. Ice Cream Field 32, 3, p. 54, March, 1938.

Viennese ice cream, according to Sidney Freier, an ice cream manufacturer of Vienna, is made with egg yolks (10 to 20 yolks for 1 quart of ice cream), milk, cream, butter and 18 per cent sugar. He claims the flavor must be very pronounced, *e.g.*, 30 to 40 per cent crushed fruit is used in Viennese fruit ice cream; the taste is never doubtful.

Coffee is the most popular flavor in Vienna followed by nougat or hazelnut. Strawberry is also a popular flavor, but vanilla and chocolate are in little demand.

Mr. Freier feels that more emphasis should be placed upon flavor in this country, but he is impressed with the magnitude speed and efficiency of American machines.

W.C.C.

**413. The Small City Plant.** M. W. YALE and R. C. HICKEY, N. Y. Agr. Exp. Sta., Geneva, N. Y. Ice Cream Field 32, 3, p. 34, March, 1938.

"In general, venders of ice cream take poor sanitary care of dippers and scoops" according to the authors. They claim further that small

retailer manufacturers are, as a group, least familiar with sanitary principles.

They state further, "the greatest need for improvement in sanitation appears to be in the dispensing of bulk ice cream in a cleaner and more sanitary manner. However, this need is no greater than that for improvement in the handling of many other foods and beverages; and it is doubtful whether ice cream should be singled out for action. The whole question of sanitation in respect to food handling is receiving and should receive much attention at the present time."

W.C.C.

- 414. The Use of Lecithin.** J. H. ERB and H. COLLINS, Ohio State Univ., Columbus. *Ice Cream Field* 32, 5, p. 42, May, 1938.

Dipping chocolate bars ordinarily results in the incorporation of some moisture in the coating which increases its viscosity and decreases the amount of surface coverage. The addition of 0.2 to 0.4 per cent lecithin tends to reduce the increase in viscosity due to water dilution.

W.C.C.

- 415. Egg Yolk as a Mix Ingredient.** C. D. DAHLE, Penn. State College, State College, Pa. *Ice Cream Field* 32, 5, p. 25, May, 1938.

The author points out that since 1920 the commercial use of egg products for ice cream manufacture has increased materially. Egg yolk improves the whipping properties of ice cream mixes especially those made with butter or frozen cream as the sources of fat. Often beneficial results are also obtained from its use in chocolate ice cream.

The various types of egg products available for use in ice cream are considered and a brief discussion presented as to the possible constituents of egg yolk which may be responsible for the improved whipping qualities of the mix.

W.C.C.

- 416. Use of Anti-Oxidants in Ice Cream.** A. C. MACK and P. H. TRACY, Dept. Dairy Industry, Univ. of Ill., Urbana, Ill. *Ice Cream Rev.* 21, 6, p. 82, Jan., 1938.

Oat flour was added to ice cream mixes containing three p.p.m. of added copper. The amount of oat flour added ranged from 0.1 to 0.5 per cent. In order to be assured of ample anti-oxidative protection in vanilla ice cream it is recommended that 0.5 per cent oat flour be added. Various methods of adding oat flour to the mix were studied. The most satisfactory methods were to add the oat flour to the mix with the sugar or to add it in dry form at the freezer. It was found more difficult to control the development of stale metallic flavor in strawberry ice cream by the addition of oat flour than in vanilla ice cream. Oat flour added at the rate of 0.5 per cent delayed the oxidized flavor development.

J.H.E.

- 417. Profitably Priced Packages Preferred.** J. H. CAROTHERS, Los Angeles, California. *Ice Cream Rev.* 21, 9, p. 44, April, 1938.

In order to give the consumer a better quality and more fairly priced package the overrun should be controlled, and a price should be set on the package which would allow a fair margin of profit, to the manufacturer and the dealer. It is suggested that the price be set on a unit basis so the dealer will know a certain profit will be made on each individual unit sale.

J.H.E.

- 418. A Study of the Qualities of Commercial Ice Cream.** W. H. BROWN, Dairy Dept., Purdue Univ., Lafayette, Indiana. *Ice Cream Rev.* 21, 9, p. 110, April, 1938.

Analytical and bacteriological results compiled on 570 samples of commercial ice cream at Purdue University are tabulated and discussed.

J.H.E.

- 419. Controlling Stale Flavors in Ice Cream.** K. G. WECKEL, Dept. of Dairy Industry, Univ. of Wis., Madison, Wis. *Ice Cream Rev.* 21, 9, p. 35, April, 1938.

State flavors in ice cream originate through the use of inferior ingredients, inadvertent equipment effects, and slow turnover of the product. Contributing factors to each of the above are discussed and precautions and remedies suggested.

J.H.E.

- 420. Ice Cream Regulations.** M. G. YOUNG, Missouri Dept. of Health. *Ice Cream Rev.* 21, 6, p. 28, Jan., 1938.

The consumer of ice cream and other dairy products is entitled to the same health protection as the consumer of a bottle of fluid milk. Factors discussed are the regulation of the raw products going into ice cream, the control problems arising since the development of counter freezers, the question of requiring pasteurizing of mix and manufacture of ice cream to be a continuous process, and the sanitary conditions of the retail outlet.

J.H.E.

- 421. Refrigeration in the Ice Cream Industry.** W. L. PHARO, York Ice Machinery Corp., Charlotte, N. C. *Ice Cream Rev.* 21, 9, p. 46, April, 1938.

The most common cause of inefficient refrigeration plant performance can be credited to high condensing pressures. An illustration is given showing the increased cost of operation due to excessive head pressure. The most common causes of high pressures are non-condensable gases in the system, dirty condensers, lack of condensing water or condensing water not cool enough, and lack of sufficient condensing surface.

J.H.E.

422. **Frosted Malted.** ANONYMOUS. *Ice Cream Field* 32, 1, p. 7, Jan., 1938.

Frosted malted milk is not ice cream, instead it is a sherbet with low butter-fat content. It has been characterized as the "drink you eat with a spoon," actually it is a semi-frozen product that is eaten with a spoon.

Special mixers are used for making this product which are illustrated.

Under the caption "How it's done" is given a formula for the manufacturer and one for the retailer. W.C.C.

Editor's Note. "This product is what is generally known as 'Ice Milk.'"

423. **Frosted Malted.** ANONYMOUS. *Ice Cream Rev.* 21, 10, p. 32; May, 1938.

A large wholesale ice cream manufacturer introduced a Frosted Malted ice milk and secured highly satisfactory results by adding milk to the product and mixing it on a fountain mixer. It is a product low in fat content and high in total solids making it an ideal hot weather drink. A formula is given for its manufacture. J.H.E.

424. **Large Scale Production of Fancy Ice Cream, Chocolate-Coated Bars and Specialties.** CHARLES WEINREICH, Cherry-Burrell Corp., Chicago, Ill. *Ice Cream Rev.* 21, 10, p. 88, May, 1938.

Consumption of ice cream novelties has increased until today about 25 per cent of all ice cream manufactured is in this form. Several methods are outlined for the production of chocolate-coated ice cream bars.

J.H.E.

425. **Routine Calculation of Ice Cream Mixes.** CARL DUNCAN. *Ice Cream Rev.* 21, 8, p. 41, March, 1938.

Instruction, using sample mixes for demonstration, is given for standardizing the ice cream mix. J.H.E.

426. **The "Big Ten" in Ice Cream Merchandising.** R. F. GILBERT, Hydrox Corp., Chicago, Ill. *Ice Cream Rev.* 21, 9, p. 96, April, 1938.

Ten specific points are suggested for successful ice cream merchandising at the soda fountain. J.H.E.

427. **Making Ice Milk Mix from Ice Cream Mix.** H. A. COLLINS, San Jose, California. *Ice Cream Rev.* 21, 10, p. 38, May, 1938.

Calculations are explained for standardizing ice milk from regular ice cream mix. J.H.E.

- 428. Ice Milk.** G. D. TURNBOW, Oakland, California. *Ice Cream Rev.* 21, 10, p. 30, May, 1938.

The author justifies the existence of ice milk, a product similar to ice cream, but containing only 4 per cent milk fat. He states it merits development and regulation because it is a tasteful confection, it has exceptional food value, and is important in the economic structure of the dairy industry. The author states that in 1936 California produced 15,664,734 gallons of ice cream and 7,190,587 gallons of ice milk. J.H.E.

- 429. Diabetic Ice Cream.** B. E. HORRELL, Dairy Dept., Purdue Univ., Lafayette Indiana. *Ice Cream Rev.* 21, 10, p. 46, May, 1938.

Two formulas for ice cream suitable for people afflicted with diabetes are given. Saccharin is the sweetener in place of sugar. J.H.E.

- 430. Seasonal Specialties.** JOHN CLAITOR. *Ice Cream Field* 32, 3, p. 42, March, 1938.

Several recipes are given for small ice cream manufacturers who are not equipped to homogenize their mixes. W.C.C.

- 431. Bacteria in the Mix.** D. LEVOWITZ, New Jersey Laboratories. *Ice Cream Field* 32, 9, p. 10, March, 1938; 32, 4, p. 23, April, 1938; 32, 5, p. 15, May, 1938.

The author attempts to describe some of the elementary principles of microbiology in very simple terminology. Different types of organisms are illustrated, some of the sources of contamination discussed and desirable practices indicated. W.C.C.

Other abstracts of interest are numbers 372, 373, 375, 447, 449, 450, 452, 455, 459, 463 and 464.

## MILK

- 432. Seasonal Changes in Market Milk Production in Pennsylvania. The Relation of Month-to-month Fluctuations in Milk Sales to Prices Received by Farmers.** F. F. LININGER and C. W. PIERCE, Pennsylvania Agric. Exper. Sta., State College, Pa. *Bull.* 358, April, 1938.

There is a wide variation in the amount of milk produced during the different seasons of the year. In many cases there is twice as much shipped in June as there is during the month of November. Records of milk shipments indicate a trend toward more variable production in the Philadelphia and Pittsburgh milk sheds following the abandonment of the base-surplus plans a few years ago. Seasonal sales of milk from farms have always

varied widely in the New York milk sheds, where no plan for the specific purpose of leveling production has ever been in general use.

The report deals with the seasonal production, distribution of sales and prices for the three large markets, New York, Pittsburgh, and Philadelphia, drawing supplies from the state of Pennsylvania.

The average per cent of milk received by these three markets during the month of November was 78.3 per cent while in June it was 131 per cent, using 100 per cent as the average for the year. In the Philadelphia market during the month of November 87.6 per cent of the milk received was used for fluid milk purposes, while in June only 58 per cent of the milk received was used for fluid milk purposes.

Because fluid milk distributors must have an adequate supply of milk during the low periods of production, they have a supply in excess of their fluid requirements during the remainder of the year as a result of fluctuating production.

The average price paid producers depends largely on the percentage of the total supply sold for fluid uses. Therefore, prices are higher in the fall and winter months than during the spring and early summer months. Production is more varied in both the Pennsylvania section of the New York milk shed and in the Pittsburgh milk shed than in the Philadelphia milk shed. Seasonal fluctuation in production in the Pittsburgh and Philadelphia sheds have increased since the discontinuance of the "basic-surplus" plans during 1933 and 1934. For all groups of producers seasonal fluctuations in production have widened since 1933.

A trend toward uneven milk production tends to widen a milk shed; to increase the cost of marketing by requiring additional investments in plants, equipment and trucking facilities necessary to handle peak summer milk supplies; and to increase seasonal fluctuations in average prices paid to producers for milk.

The present price system in Pennsylvania favors uneven production. For this reason many producers have been demanding a change in price policies which would favor even production. W.D.S.

**433. The Use of Citric Acid and Sodium Citrate in Milk and Milk Products.** HUGH L. TEMPLETON, Univ. of Wisconsin, Madison, Wis. Wis. Agric. Exper. Sta. Res. Bull. 133, Nov., 1937.

Judges preferred those starters containing either citric acid or sodium citrate. The addition of citric acid or sodium citrate to the starter culture or to the cream to be used for butter-making, or both, seems to give a butter with a more pronounced flavor and aroma. The addition of two to six ounces of sodium citrate per thousand pounds of cream is effective for preventing feathering of cream because it counteracts the effect of calcium salts in either the water or the cream. The use of limited amounts of sodium citrate in

cream tends to minimize the cream plug defect and although large amounts completely eliminate cream plug, the flavor of the cream is affected adversely. Experiments with whipping cream show the addition of sodium citrate decreases the whipping time when the fat content of the cream is less than 35%. Other factors influencing the whipping of cream, such as fat content, temperature of pasteurization and the like were studied experimentally. Brief comments indicate the possible application of sodium citrate for stabilizing condensed milk, for aiding the ripening of natural cheese and for the production of soft-curd milk. Citric acid may be used advantageously in milk for infant feeding under a doctor's direction. W.V.P.

- 434. Base and Surplus Plans Level Out Milk Production.** R. W. SHERMAN, Ohio Agric. Exper. Sta., Wooster, Ohio. Ohio Exper. Sta. Weekly Press Bull. No. XXIII-19, July 14, 1938.

Base and surplus plans in four Ohio markets have been an influence in leveling out the yearly supply of milk. In some instances the difference between the flush and shortage months was 10 pounds less per day than before the plan was in operation. W.E.K.

- 435. Evaluation of Methods of Determining the Efficiency of Pasteurizing Milk.** E. H. PARFITT, Univ. of Purdue, Lafayette, Indiana. Ann. Conv. Intern. Assoc. Milk Dealers, Laboratory Section, p. 183, 1937.

The value of comparison of laboratory and commercial pasteurization, line testing, line bottle testing and coliform content is discussed and some figures given showing how each criterion is used. The author states the coliform content is one of the best. E.F.G.

- 436. The Causes of Off-flavors in Milk; the Facts and a Theory.** J. A. ANDERSON, S. T. WILSON, and J. G. HARDENBERGH, Rutgers Univ., New Brunswick, New Jersey. Ann. Conv. Intern. Assoc. Milk Dealers, Laboratory Section, p. 177, 1937.

The authors' theory is that the oxidized, rancid, flat and insipid flavors which develop in milk of low bacterial content have their origin in a carotene deficiency in the ration. This effects the milk directly by reducing its carotene content and what appears to be more important causes a vitamin A deficiency in the cow which results in an abnormal distribution of enzymes in blood and milk. This accounts for excessive amounts of lipase found in milk which develops a rancid flavor. E.F.G.

- 437. Plant Processing and Control Methods in Preventing Oxidized Flavor.** W. H. MARTIN, Kansas State College, Manhattan, Kansas. Ann. Conv. Intern. Assoc. Milk Dealers, Laboratory Section, p. 169, 1937.

Several treatments which have at times been suggested for preventing the flavor are mentioned. Factors on the production side which cause milk to be susceptible are listed. In the plant metallic contamination, cleaning and sterilizing and the effect of light at all points in processing and delivery is stressed. Discarding the first milk through the equipment is recommended.

E.F.G.

438. **Variations in Susceptibility of Milk as Secreted by the Cow.** E. O. ANDERSON, Connecticut State College, Storrs, Connecticut. Ann. Conv. Intern. Assoc. Milk Dealers, Laboratory Section, p. 153, 1937.

It is known that winter milk is higher in acidity and also more readily develops oxidized flavor than summer milk. Results on seven trials of .19% acidity milk compared with 7 trials of this milk reduced in acidity to .15% with  $\text{Na}_2\text{CO}_3 \cdot 5\text{H}_2\text{O}$  gave strong oxidized flavor in 4 of the former and only 2 of the latter samples at the end of 72 hours. Milk from different sources seems to vary in the reduction of acidity needed to inhibit oxidized flavor. The theory is advanced that the change in pH upon reduction in acidity has an effect upon enzymes secreted by the cow; that the cause and prevention of oxidized flavor is intimately associated with the nutrition of the animal.

E.F.G.

439. **Off Flavors in Raw and Pasteurized Milk.** G. M. TROUT, Michigan State College, East Lansing, Michigan. Ann. Conv. Intern. Assoc. Milk Dealers, Laboratory Section, p. 131, 1937.

The flavors of 920 cans of raw milk on a hot August day are reported as follows:

Flavor	No. of cans	% distribution
Clean and pleasant .....	413	44.89
Feed .....	219	23.80
Musty .....	101	10.97
High acid .....	73	7.93
Unclean .....	58	6.30
Barny .....	13	1.41
Cowry .....	12	1.30
Oily .....	13	1.41
Miscellaneous .....	18	1.95
Total .....	920	99.96

Flavors in the pasteurized milk of 22 dealers during September and October are given as follows:

Flavor	Percentage distribution	
	Fresh 1 day	After 3 days
Clean, pleasant . . . . .	13.3	12.0
Cooked or heated . . . . .	65.5	30.9
Oxidized . . . . .	5.5	20.7
Barny . . . . .	4.4	.....
Cowy . . . . .	3.3	.. ..
Metallic . . . . .	1.1	5.2
Unclean . . . . .	1.1	9.6
High acid . . . . .	1.1	.. ..
Stale . . . . .	.....	10.3
Flat . . . . .	.. ..	10.3
Sour . . . . .	.....	1.7
Total . . . . .	99.7	99.7

Some flavors in raw bottled milk, homogenized and irradiated milk are mentioned. E.F.G.

**439a. Theoretical Aspects of the Cause of Oxidized Flavor Particularly from the Lecithin Angle.** L. M. THURSTON, Univ. of Florida, Gainesville, Florida. Ann. Conv. Intern. Assoc. Milk Dealers, Laboratory Section, p. 143, 1937.

The author suggests the following classification of milks from the standpoint of oxidized flavor:

1. Spontaneous milk: Milk which is capable of developing oxidized flavor spontaneously, *i.e.*, without the presence of iron or copper as a contaminant.
2. Susceptible milk: Milk which does not develop oxidized flavor spontaneously, but is susceptible in that contamination with copper or iron will cause development of the flavor.
3. Non-susceptible milk: Milk in which oxidized flavor cannot be produced by the addition of copper or iron.

The theory of an oxidizing enzyme in "spontaneous milk" of the catalytic effect of Fe and Cu in susceptible milk and the presence of increasing proportions of reducing substances in non-susceptible milk is discussed. Vitamin C is important in the latter instance.

Attention is called to the results obtained by various investigators to indicate the possibility of lecithin being the material oxidized in the case of "susceptible" milk, whereas in spontaneous milk it is both butterfat and lecithin-cephalin. The most likely present theory to explain the non-appearance of oxidized flavor in homogenized milk is the increased adsorption of protective protein on the surface of the fat globule.

Some reduction of the development of the flavor in milk agitated or frozen may possibly be explained by a reduced fat surface. "Spontaneous" milk heated to 165 to 168° F. probably results in destruction of the enzyme. Sun-

light alone is an oxidizing agent and the flavor produced differs from the so-called oxidized flavor found in "spontaneous milk" or caused by copper in susceptible milk. E.F.G.

- 440. The Resazurin Test for Sanitary Condition of Milk.** G. A. RAMSDALL, Bureau of Dairy Industry, U. S. D. A., Washington, D. C. Ann. Conv. Intern. Assoc. Milk Dealers, Laboratory Section, p. 3, 1937.

The resazurin test is found to be more rapid than the methylene blue test, and to be more sensitive to the reducing influences of pathological milks and physiologically abnormal milks. With the short incubation period of one hour the flora approximates more closely the initial flora than when longer incubation periods are used. The rate of change of color from blue to pink over several hours of incubation gives considerable information relative to the types of flora existing in the milk. E.F.G.

- 441. The Practical Value of the Phosphatase Test in Determining the Efficiency of Pasteurization.** F. W. GILCREAS and W. S. DAVIS, New York State Dept. of Health, Albany, N. Y. Ann. Conv. Intern. Assoc. Milk Dealers, Laboratory Section, p. 34, 1937.

The details of the procedure and reagents used for the phosphatase test are described. Variation in incubation time from 24 hours to 18 hours had no significant effect upon the accuracy of the results. Reagents prepared commercially and buffer substrate tablets were found satisfactory. The phenol concentrations corresponding to various degrees of treatment have been established. Variations of 5 minutes or greater in heating time were readily distinguished and the addition of .1% or more of raw milk gave results indicating incomplete pasteurization. Variations in temperature were also easily detected. High temperature process pasteurization could also be checked. Application of the technique to 780 samples collected from delivery trucks and labeled pasteurized, detected the treatment of the milk correctly in 96% of the samples. E.F.G.

- 442. Determining the Efficiency of Milk Pasteurization.** P. H. TRACY and A. J. HAHN, University of Illinois, Urbana, Illinois. Ann. Conv. Intern. Assoc. Milk Dealers, Laboratory Section, p. 57, 1937.

After a fairly comprehensive review of the literature with reference to the phosphatase test including an explanation of the principles involved the author suggests several aspects of the test which require further study. The Scharer modification of the phosphatase test devised by Kay and Graham is described in detail and was used in the work reported in securing the following results. The authors report attempts to improve Scharer's method by the application of the photoelectric cell in determining the degree of the

phenol color formed, thus making it possible to make more accurate determinations of the amount of phenol present. The principle and construction of the photoelectric cell is given in detail. The photoelectric cell made possible the detection of as low as .1% raw milk. Differences in holding time were more difficult to detect at 145° F. than at 142° F. In either case a raw sample which could be pasteurized under controlled conditions is needed as a reference sample. Corrosive sublimate tablets were found to be usable for preserving milk to be examined by the phosphatase test. E.F.G.

- 443. Dairymen Must Keep Milk Cold.** L. H. BURGWARD, Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Exp. Sta. Weekly Press Bull. No. XXIII-4, March 31, 1938.

Examinations of the milk sent into Columbus by 10 dairymen showed that during February and March there was a progressive increase in the temperature of the milk and in the bacterial count. This experience points to the necessity for careful cooling at all seasons of the year. W.E.K.

- 444. Milk Trucking Interests Grow.** C. G. McBRIDE, Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Exp. Sta. Weekly Press Bull. No. XXIII-5, Apr. 7, 1938.

Because the trucking operation represents approximately 10 per cent of all the costs of marketing fluid milk, more economic control of this process is indicated. W.E.K.

- 445. Make Survey of Family Milk Use.** HUGHINA MCKAY, Ohio Agr. Sta., Wooster, Ohio. Ohio Exp. Sta. Weekly Press Bull. No. XXIII-8, Apr. 28, 1938.

A survey of 28,966 families in 59 cities revealed that the average weekly consumption of milk was 2.44 quarts; that less than 1 quart per person weekly was purchased by 4,126 families; that 529 of these families bought no milk. W.E.K.

- 446. A Study of Causes of Damage to Cap, Seat and Bead of Comalac Universal Milk Bottles.** MARCUS DEY, Golden State Co., Ltd., Los Angeles, California. Ann. Conv. Intern. Assoc. Milk Dealers, Plant Section, p. 21, 1937.

This comprehensive study was undertaken at the direction of Comalac, reported to be the largest purchaser of milk bottles in this country, to discover the causes of so much chippage on the tip and bead of Comalac bottles. Some of the results are:

1. 2.9 per cent of new quarts received from the factory were defective.
  2. Differences in chipping were observed in ware from different plants.
- "Over pressed" cap seats were a contributing factor.

3. Two wire cases are a definite cause of bead chippage and their use should be discontinued.

4. Bottle washing operations cause extreme damage to bead and cap seat. Comparisons of washers are given and suggestions for operation to reduce injury to bottles.

5. Corrosive bottle washing materials cause spalled glass and is the forerunner of etching and chipping. The solubility of glass in various washing materials is given. The least solubility, .878 per cent, was obtained with a mixture of flake caustic 50 per cent and sodium metasilicate 50 per cent.

6. Capping machines do not damage the cap seat.

7. Significant damage to the bead or finish resulted when filled bottles were cased 4 at a time but not when cased 2 at a time.

8. Only 1.6 per cent of bottles were damaged on the bead when in the hands of retail customers.

9. Investigation of handling and transporting of cased bottles deserves to reduce damage. Specific recommendations are given. E.F.G.

**447. How Milk is Produced and Distributed in the Argentine.** W. BOB S. FELS, Sales Mgr. of the farm-ranch, "Estancia Tatay," Buenos Aires, Argentine. *Milk Dealer* 27, 5, p. 40, Feb. 1938.

A description of the milk industry in the Argentine, especially as carried on by the farm-ranch Estancia Tatay. This farm-ranch has approximately 15,000 head of cattle and the milk is pasteurized on the farm where it is produced. C.J.B.

**448. Preventing the Oxidized Flavor in Milk and Milk Products.** C. D. DAHLE, Dairy Dept., Penn. State College, State College, Pa. *Milk Dealer* 27, 5, p. 68, Feb. 1938.

A report of experimental work with oxidized flavor in milk. The author draws the following conclusions: 1. There are numerous means for delaying or preventing the onset of flavors in dairy products that are of an oxidative nature. 2. In milk, in which the flavor develops spontaneously, high temperature (170° F.), homogenization, nitrogen replacement of the free oxygen, increasing bacterial population, feed and anti-oxidants are effective. 3. Certain anti-oxidants in other fats are pro-oxidants in milk fat. 4. An anti-oxidant contained in oat flour proved to be particularly efficient. C.J.B.

**449. The Phosphatase Test for Determining Efficiency of Pasteurization.** E. H. PARFITT. *Milk Plant Mo.* 27, 1, p. 34, Jan. 1938.

The author gives a comprehensive summary of the phosphatase test, the principles involved, the methods used, applications and precautions. G.M.T.

**450. The Use of Chlorine Disinfectant in the Sterilization of Dairy Equipment.** LEWIS SHERE. *Milk Plant Mo.* 27, 1, p. 42, Jan. 1938.

Methods of sterilizing dairy equipment are given with special reference to the selection and use of chlorine, pointing out the differences in the corrosive action of chlorine sterilizers. G.M.T.

**451. Now is the Time and Here is the Way to Tackle the Fly and Insect Problem.** E. M. SEARLS, FRED M. SNYDER, and C. L. FLUKE. Milk Plant Mo. 27, 4, p. 32, April 1938.

Precautions to observe in the selection and use of sprays are given as well as types of sprayers to use and methods for testing them. Life cycles of some insects are presented. A number of control measures are discussed. G.M.T.

**452. Daylight Versus Night Delivery.** F. E. ROGERS. Milk Plant Mo. 27, 3, p. 28, March 1938.

A discussion of the possible changes in systems of milk delivery giving the advantages and disadvantages of the daylight delivery system. G.M.T.

**453. What Makes Milk Stone, How to Prevent It and How to Eliminate It.** H. A. RUEHE. Milk Plant Mo. 27, 4, p. 30, April 1938.

The composition of milk stone is dependent somewhat upon the product, ice cream mix, cream, skim milk, or whole milk, from which it is precipitated. Generally formed by the precipitation of calcium, sodium, and magnesium phosphate by heat, it contains also some entrapped fat. Analysis of milk stone from a vat coil used for pasteurizing ice cream mix at 160° F. showed the milk stone to contain 43.54 per cent protein, 42.03 per cent ash and 12.44 per cent ether extract (fat); whereas holder pasteurized whole milk stone contained 31.19 per cent protein, 5.62 per cent ash and 42.65 per cent ether extract; and 180° F. skim milk stone had 37.82 per cent protein, 52.6 per cent ash and 6.08 per cent ether extract. Prevention of milk stone formation may be accomplished (1) by proper adjustment of the heating medium during pasteurization (2) by use of non-film-forming washing compounds with hard water and (3) by rinsing with cold water rather than hot when the water is hard. Equipment may be freed from milk stone deposits by use of  $\frac{1}{16}$  of 1 per cent tartaric acid or by use of commercial compounds especially prepared for that purpose. G.M.T.

**454. The Action of Sunlight on Milk.** LASCAR BURUIANA, Dept. of Vet. Med., Univ. of Bucharest. Biochem. J. 31: 1452, 1937.

The influence of various factors responsible for the reduction of methylene blue in milk exposed to sunlight has been reviewed experimentally, and certain phenomena distinguished.

Two effects are noted:

(1) Oxidation of unsaturated fat. This phenomenon is independent of the decoloration of methylene blue. The reduction of methylene blue is, however, aided by this oxidation of the unsaturated fat, which produces anaerobic conditions in the milk by using up the dissolved oxygen and thus allows the second phenomenon to appear.

(2) Oxidation by catalytic dehydrogenation of the ascorbic acid present in the milk. This dehydrogenation is responsible for the decoloration of the methylene blue, which serves as hydrogen acceptor. When all the ascorbic acid has been oxidized, the color of the methylene blue is restored if air or oxygen be admitted. The determination of the substances oxidizable by iodine before and after exposure to sunlight can be used to evaluate the vitamin C content of milk. The results of this method agree well with those obtained by direct titration with 2:6-dichlorophenolindophenol by the Schlemmer method. The rate of reduction of methylene blue on exposure of milk to sunlight does not give quantitative information of the content of ascorbic acid because this rate depends on the amount of unsaturated fat present, which plays a part described above as oxygen absorption. With the exception of mare's milk, the milks examined did not contain reduced glutathione.

K.G.W.

**455. Irradiation of Milk. Factors Affecting Antirachitic Response.** H. H. BECK, H. C. JACKSON and K. G. WECKEL, Univ. of Wisconsin, Madison, Wis. *Ind. & Eng. Chem.* 30: 632-639, 1938.

A study was made of factors affecting the efficiency of commercial irradiating equipment in the photochemical synthesis of vitamin D in fluid and evaporated milk exposed in flowing films. A high-pressure, air-cooled quartz mercury-vapor arc provided the radiation, the intensity of which was measured, recorded and maintained automatically. The rate of flow of the milk and the width of film on a vertical, stainless steel surface was controlled while the elevation and distance of the arc from the milk film was adjustable. The variations in radiant energy impinging on the film depend on the solid angle of radiation intercepted by the surface. This is conceived as a rectangular pyramid with apex at the center of the arc and base delineated by the limits of the rectangular surface. To determine the most suitable solid angle, the film travel distance, length of vertical angle, and film width of horizontal angle, were independently varied. The effectiveness of the irradiation was measured by bioassay methods.

Distances of the arc of 4, 6, 8 and 10 inches from the center of the film were used with emission rates producing intensities in wave lengths of less than 3000 Å of 1000, 2000, 4000 and 6000 microwatts measured at a distance of 8 inches horizontally opposite the center of the arc. The plan permitted the variation of radiation intensity while applying a constant amount of energy within the fixed solid angle of radiation. Experiments

were conducted with four rates of milk flow with each of four rates of energy emission at each of four distances from the arc. Both fresh milk and homogenized evaporated milk were studied.

The effect of varying the horizontal and vertical angles was found to be an increase of vitamin D potency with an increase in vertical angle of radiation up to  $100^\circ$  and a decrease in potency as the horizontal angle is widened beyond  $60^\circ$ . Hence, the effective solid angle of radiation for these experiments was defined as one having  $90^\circ$  vertical and horizontal angles. Limiting the applied radiations in this manner, 6 inches proved to be the optimum distance between arc and film for maximum potency, independent of intensity and film capacity. It is assumed that other little-known factors are involved in determining the effectiveness of this 6 inch distance.

The vitamin D potency bears a parabolic relationship to the amount of radiant energy applied. Analyses of the data reveal that, for any given distance between arc and film, identical variations in the amount of applied energy, however produced, have identical effects measured in terms of vitamin D potency of the milk. This holds true even for successive exposures of the milk. This leads to the generalization that irradiation of flowing films of milk with a given radiation source at a given distance from the film produces an antirachitic potency dependent upon the number of successive exposures, the film capacity, and the radiation intensity, only insofar as these affect the amount of applied energy.

From their data the authors derive a mathematical expression for the potency-energy relations as follows:

$$P = KA^{1/n} \frac{I^{1/n}}{Q^{1/n}} \text{ where}$$

I = intensity  
A = area of milk film  
Q = quantity discharge per unit time  
K = a constant  
1/n = decimal exponent in equivalent fraction,  
ranging between 0.5 and 0.6

Allowing for maximum deviations of 10 per cent in I and Q, the variations in potency of the milk will lie within the limits of accuracy of the bioassay method.

Since the film capacity-potency relation is hyperbolic and the intensity-potency relation is parabolic, it is desirable to employ sufficiently high intensities and film capacities to avoid excessive variations in potencies. Present commercial equipment satisfies the limitations developed by these investigators.

J.H.N.

Other abstracts of interest are numbers 372, 374, 375, 376, 379, 393, 394, 395, 396, 397, 398, 399, 401, 403, 459, 463 and 464.

## MISCELLANEOUS

- 456. Cold Storage Locker Plants.** MARVIN A. SCHAARS. Univ. of Wisconsin, Madison, Wis. Nat. Butter and Cheese Jr. Article I, 29, 11, p. 6, June 10th, 1938; Article II, 29, 12, p. 6, June 25, 1938.

Article I. About 90 per cent of approximately 2,000 cold storage locker plants have been built since 1936. They are operating in northern and western states chiefly, but are reported in at least twenty-one states. Some provide cold storage space only; others furnish a butchering service which includes butchering, chilling, cutting, wrapping, grinding, smoking, curing, and the like. The modern plant provides rooms for chilling, ageing, cutting, sharp freezing and lockers. Investment in refrigerating machines, insulation, lockers and equipment fully installed, and for land and building total approximately \$25.00 to \$35.00 per locker in plants with 200 to 500 lockers. Limited information on cost of operation makes it possible to estimate an approximate cost per year of \$11.46 and \$10.62 per locker in complete service plants of 300 and 500 locker capacities, respectively. This cost is exclusive of interest on investment. Earning capacities of locker plants are discussed and estimates are shown that suggest a net return on the investment approximately 10 per cent.

Article II. Locker plants may be licensed by the state in which they operate. Plants may be operated in conjunction with some other enterprise, such as cheese factories, creameries, ice plants, meat markets and grocery stores. Locker plants may be either privately or cooperatively owned and operated. An ideal location is a thriving small town up to 5,000 population which is surrounded by a thickly populated farming area. Successfully operated city plants indicate that operations are not restricted to rural communities. Existing plants draw 75 to 85 per cent of their patronage from farmers. The permanency of the locker system can be assured if it provides an economical, attractive service that fits in with the modern household economy.

W.V.P.

- 457. A Trial with Temporary Silos.** W. E. KRAUSS, C. C. HAYDEN, A. E. PERKINS and R. G. WASHBURN. Ohio Agric. Exper. Sta., Wooster, Ohio. Ohio Bimonthly Bull. Vol. XXIII, No. 192, May-June, 1938.

Second cutting alfalfa was put up in temporary silos of the snow fence type. The material in one silo was untreated; molasses at the rate of 2 per cent was added to that of a second; and the A. I. V. treatment was applied to the alfalfa put into a third silo. Much spoilage occurred in all three silos, particularly at the top and around the edges. Carotene preservation was best in the molasses-treated material. In a palatability trial cows preferred corn silage to alfalfa silage. The carotene content of the milk pro-

duced varied in accordance with the carotene content of the silage. Suggestions and precautions concerning the use of temporary silos are given, chief of which is that temporary silos are probably best adapted to storing a late crop for feeding in the fall or early winter to supplement and extend the use of a permanent silo.

W.E.K.

- 458. Protein Production Increased by Liming.** E. E. BARNES, Ohio Agric. Exper. Sta., Wooster, Ohio. Ohio Exper. Sta. Weekly Press Bull. No. XXIII-18, July 7, 1938.

A 3-year rotation of corn, small grain, and hay (red clover, mammoth clover, alsike clover, sweet clover, alfalfa, soybeans, or timothy) was followed on soil limed to the neutral point and on unlimed soil with a pH of 5.0. On an average, liming to the neutral point increased the yield of dry matter 77 per cent and the protein 112 per cent.

W.E.K.

- 459. A. I. V. Silage.** H. T. GREENE and H. OTTERSON, Brook Hill Farm, Genesee Depot, Wis. Cert. Milk 13, 144, p. 9, April 1938.

This paper deals largely with a review of recent investigations as to values found in A.I.V. silage. The following subjects are discussed in connection with A.I.V. silage: Grass juice factor, use of different acids in the manufacture of carotenoids, and proteins and nitrogen compounds.

W.S.M.

- 460. Eleventh World's Dairy Congress.** A. C. DAHLBERG, New York Exp. Sta., Geneva, New York. Ann. Conv. Intern. Assoc. Milk Dealers, Laboratory Section, P. 79. 1937.

The author reports an attendance of 3,760 from 53 countries of which slightly less than half were from Germany, the congress meeting in Berlin. Seventy-two registered from U. S. A brief digest is given of many of the noteworthy papers, particularly those which dealt with milk from the standpoint of sanitation, disease control, composition and flavor, quality control, nutritive value and methods of processing. It was noted that the reports dealt largely with problems under European conditions.

E.F.G.

- 461. Cooperative Experiments in Pasture Improvement.** D. R. DODD, Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Bimonthly Bull. 23, No. 191: p. 39, 1938.

This is a progress report of results obtained from 1931 through 1937 in experiments with fertilizers on pasture situated at many points throughout Ohio. Best yields of both total and desirable herbage were obtained when combinations of superphosphate and sulfate of ammonia, or superphosphate, muriate of potash, or sulfate of ammonia were used. Equivalent production and returns also favored these treatments.

The relative amount of white clover in the pasture was found to influence

herbage yield and possible returns. Phosphate alone lowers the cost and greatly increases the returns where a high clover content can be maintained. Nitrogen in addition has little effect on returns and greatly increases the cost. when clover can not be grown the addition of nitrogen is effective.

These tests are cited as evidence to justify the general improvement of adapted permanent pasture lands. W.E.K.

- 462. Management Factor in Pasture Improvement.** D. R. DODD, Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Exp. Sta. Weekly Press Bull. XXIII 4, March 31, 1938.

Experiments in which different heights and frequency of clipping were practiced showed that short, frequent clipping affected adversely the yield and character of the herbage. Controlled grazing, mowing, or both, are suggested for keeping the growth of herbage under control. W.E.K.

- 463. Can Make Good Silage of Certain Hay Crops.** Dairy Dept., Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Exp. Sta. Weekly Press Bull. No. XXIII-8, Apr. 28, 1938.

Good silage can be made from such hay crops as alfalfa, clover, and soybeans, or mixtures of these with grasses or corn. The use of molasses for legumes is recommended. W.E.K.

- 464. Industry's Responsibility in Labor Relations.** C. A. JAY, Dallas Open Shop Association. Ann. Conv. Intern. Assoc. Milk Dealers, General Sessions, p. 83, 1937.

The effects of the National Labor Relations Act in prevention of strikes which is stated as one of its objects, can be judged by the fact that in the four years, 1933-1936 inclusive, the number of workers involved in strikes was 299 per cent greater than in the four years, 1929-1933 inclusive, and the number of man-days lost was 152.5 per cent greater in the latter period. Ignorance of the National Labor Relations Act and disregard of its provisions has resulted in confusion. Every employer should know what the act requires and what it does not. The writer proceeds to explain many of the more important provisions of the Act as they specify employer-employee relations and what each can and cannot do. No single piece of legislation heretofore proposed, has had such far-reaching potential consequences as that to fix wages and hours of labor in American industry.

The author states that he believes such a law is undesirable because it is impossible of accomplishment. He then outlines the basis of such a law if it is granted that it is inevitable. He recommends first that labor problems be a major subject for study by the Association and second, that a better understanding between employer and employee be developed. The milk industry cannot indiscriminately increase the cost of its product in order to

meet wage scales which are out of line with the earnings of the average customer of this industry. E.F.G.

- 465. Getting Maximum Results from the Refrigerating System.** H. A. RUEHE, Dept. of Dairy Husbandry, Univ. of Ill., Urbana, Ill. *Ice Cream Rev.* 21, 10, p. 104, May 1938.

Some of the factors which affect efficiency in refrigerating machinery and which any operator can remedy are given. J.H.E.

- 466. Storage Lockers for Revenue.** ANONYMOUS. *Ice Cream Field* 32, 5, p. 20, April 1938.

It is pointed out that many cold storage plants now provide rented lockers in which tradesmen and others may store perishables.

The opportunity of supplying this service in smaller towns is open to many ice cream manufacturers, milk dealers, creamery men and cheese factory operators equipped with cold storage facilities. W.C.C.

## PHYSIOLOGY

- 467. The Effect of Sympathectomy on Gestation and Lactation in the Cat.** F. A. SIMEONE and J. F. ROSS, Department of Physiology in the Harvard Medical School. *Am. J. Physiol.* 122: 3, 659, 1938.

Observations are reported on gestation, parturition and lactation in totally and partially sympathectomized cats shortly after completion of the surgical procedures and a year later. The incidence of abortions is high in animals that become pregnant shortly after sympathetic denervation of the internal genitalia. The incidence of stillbirths is high in animals that become pregnant long after sympathectomy. Sympathetic denervation of the mammary glands caused definite variation from normal functioning activity, recognizable histologically, in only 1 out of 7 pregnancies that went to term.

D.L.E.

- 468. The Effect of Thyroxin on the Carbohydrate Metabolism of Hypophysectomized Rats.** JANE A. RUSSELL, Institute of Experimental Biology, Univ. of California, Berkeley. *Am. J. Physiol.*, 122: 3, 547, 1938.

Thyroxin substitution therapy in hypophysectomized rats can restore the rate of absorption of glucose from the intestine completely to normal. The dose of crystalline thyroxin necessary for this action is less than that required to restore the metabolic rate. This treatment does not improve the maintenance of carbohydrate stores during the fasting or change the proportionate disposition of absorbed glucose in hypophysectomized rats. D.L.E.

- 469. Pancreatectomy in the Goat.** F. D. W. LUKINS and GEORGE S. COX, Medical Research Institute, Univ. of Pennsylvania, Philadelphia. *Am. J. Physiol.* 122: 729, 1938.

Four young goats were depancreatized. Following pancreatectomy, the goat has a mild type of diabetes as measured by the extremely low glycosuria and low, although increased, nitrogen excretion. Unlike the cat, dog and pig, ketonuria is slight or absent.

Explanations are offered to explain why depancreatized goats manifest a certain ability to utilize carbohydrate, as shown by two animals in which glucose was given intravenously, and only 25 to 50 per cent excreted. Even when the sugar was given by stomach tube, no sugar could be aspirated a few hours later. The assumption is made that glucose is fermented or otherwise transformed in the herbivorous stomach and absorbed in some non-reducing form. D.L.E.

- 470. Factors Determining Voluntary Ingestion of Water in Normals and in Individuals with Maximum Diabetes Insipidus.** CURT P. RICHTER, Psychobiological Laboratory, and Henry Phipps Psychiatric Clinic, Johns Hopkins Hospital, Baltimore, Md. *Am. J. Physiol.* 122: 668, 1938.

The voluntary water intake of normal animals and humans was found to be a function of surface area rather than of body weight, and hence must be a function of metabolism. The average daily intake per square meter body surface varied from 1,050 cc. to 1,238 cc. in rats, cats, dogs, monkeys, and humans, averaging 1,142 cc.

The maximum voluntary water intake in animals with diabetes insipidus was found to be a function of body weight rather than of body surface. The regulatory action of the posterior lobe secretion on the maintenance of the water balance is described. It was suggested that the level of the maximum intake with diabetes insipidus might be determined by the maximum capacity of the kidney or by the maximum capacity of all the cellular spaces of the body, both of which would be functions of body weight. D.L.E.

- 471. The Effects of Inanition on Temperature Regulation.** GEORGE CLARK, Institute of Neurology, Northwestern Univ. Medical School. *Am. J. Physiol.* 122: 646, 1938.

Normal well-nourished cats when placed on an inadequate diet react normally to cold until the weight loss considerably exceeds 30 per cent. The rectal temperature of cats, which have lost considerably more than 30 per cent of their original weight as a result of an inadequate diet, drops to extremely low levels upon exposure to cold.

The responses to heat are not interfered with by weight losses which cause abnormal responses to cold. D.L.E.

- 472. A Simple Apparatus for Milking Small Laboratory Animals.** P. L. TEMPLE and J. K. KON, National Institute for Research in Dairying, Univ. of Reading. *Biochem. J.* 31: 2197, 1937.

A diagrammatic discussion of the instrument is presented. K.G.W.

- 473. The Utilization of Lactic Acid by the Lactating Mammary Gland.** W. R. GRAHAM, JR. Dept. of Dairy Husbandry, Univ. of Mo., Columbia, Mo. *J. Biol. Chem.* 122: 1, 1937.

The experiments show that considerably more lactose was being secreted than could be accounted for by the removal of glucose from the blood and that other carbohydrate-forming compounds must be taken up by the gland. Glucose, lactic acid, and amino nitrogen are removed from the blood in substantial amounts. Eighty-five per cent of the lactose formed during the experimental period could be accounted for theoretically from glucose and lactic acid from the blood, the remaining 15 per cent may be accounted for if the amino nitrogen removed from the blood is calculated as a 3-carbon amino acid, which may be converted into lactic acid. Because experiments indicate lactic acid is produced rather than absorbed by the gland, this acid may be an important precursor of lactose of milk. K.G.W.



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## SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland	Prussian Dairy Research Institute, Kiel, Germany
International Association of Ice Cream Manufacturers	State Agricultural Colleges and Experiment Stations
International Association of Milk Dealers	The Royal Technical College, Copenhagen, Denmark
National Institute for Research in Dairying, Reading, England	United States Department of Agriculture
New York Association of Dairy and Milk Inspectors	

# ABSTRACTS OF LITERATURE

## BACTERIOLOGY

- 474. Prevalence and Classification of Hemolytic Streptococci in Pasteurized Milk.** LAWRENCE W. SLANETZ, New Hampshire Agric. Exper. Sta., Durham, N. H. Tech. Bull. 70, January, 1938.

Routine analysis of the bacterial content of various samples of milk indicated that large numbers of weakly hemolytic streptococci were often present. In the present study an attempt was made to develop suitable methods and media for the detection and isolation of these streptococci and to determine their prevalence in pasteurized milk, their origin, and their identity.

These weakly hemolytic streptococci were absent in the pasteurized milk from only two of the nine dairies studied. Counts ranged from 200 to 270,000 per cc., and in nearly all cases were greater than the standard plate counts.

Sheep blood agar proved best suited for the detection and isolation of these bacteria. No apparent growth occurred on standard meat extract agar. Studies indicated the presence of the hemolytic streptococci in the raw milk before it reached the dairies, principally due to utensils which were not cleaned and sterilized thoroughly.

Sixty strains, classified into five groups, were identified and studied in detail. All were able to resist pasteurization temperatures and to produce colonies of the alpha prime type on blood agar.

Large numbers of these streptococci in milk are undesirable, and methods should be used for their detection and elimination. K.S.M.

- 475. Induced O/R Potentials, Rates of Growth and the Volatile Acid Production of Lactic Acid Bacteria in Milk.** J. G. DAVIS, National Institute for Research in Dairying, University of Reading, Shinfield, Nr. Reading, England. Jour. Dairy Research 9: 85, 1938.

The abilities of type cultures of lactic acid bacteria to reduce five O/R indicators (methylene blue, Janus green, litmus, neutral red and safranin) were determined in plain milk and yeast dextrose milk. It is suggested that in some cases the reduction or non reduction of a dye may be used for identification purposes. Methylene blue, litmus and safranin may be of some value for this purpose. There was a marked correlation between rapidity of growth in milk, ability to reduce O/R dyes and the proportion of by-products. This relationship holds not only for the different groups and types, but also for variations in behavior observed in species kept under laboratory observation. It is suggested that rate of growth is fundamen-

tally related to the amount of by-products and hence presumably to flavor producing substances such as diacetyl and acetic acid. Thus in cheese ripening enforced slowness of growth may be as important as the presence of specific types. S.T.C.

**476. The Resazurin Test—A Preliminary Study.** J. M. FRAYER, Vt. Agric. Exper. Sta., Burlington, Vt. Bull. 435, June, 1938.

Preliminary trials were conducted with this test on samples of milk as delivered at creamery receiving stations and of individual cows' milks, drawn as aseptically as possible. Comparisons were made with the methylene blue reduction test and with plate and cell counts. The degree of dependability, the effect of variation in dye concentration, and of light and cooling were also studied. All samples were tested using one milliliter of a .005 per cent solution of resazurin to 10 ml. of sample and one-hour incubation at  $37 \pm 1^\circ \text{C}$ . ( $98.6^\circ \text{F}$ .).

The resazurin test:

1. Correlated poorly with the methylene blue reduction test, hardly at all with the plate count, and fairly well with the cell count of near-aseptically drawn milk.

2. Is sensitive to the presence of cells, to the presence and activity of bacteria, and extremely so to either strong sunlight or artificial light.

It further appears that:

3. Slight variations in dye concentration exert little effect upon the readings.

4. Cooling to and holding at low temperatures definitely retards reduction.

5. Supplementary microscopic examination is advisable.

6. Especial consideration should be given the selection of a point in the range of color changes as a deadline between samples needing and not needing further inspection.

7. The resazurin test has value if properly conducted and intelligently interpreted, but it will not likely supplant the methylene blue reduction test for testing raw milk, unless the microscope and microscopist are available to afford diagnostic assistance. J.M.F.

## CHEESE

**477. Alcohol-Glycerol Rennet Preparations in Cheese-Making.** J. G. DAVIS, National Institute for Research in Dairying, University of Reading, Shinfield, Nr. Reading, England. Jour. of Dairy Research 9: 80, 1938.

Brine rennet and alcohol-glycerol rennet extracts were compared in bacterial content, in keeping quality and in suitability for use in cheese making.

Brine rennets were found to have a bacterial count as high as 100,000 per ml., whereas the alcohol-glycerol rennets were either sterile or had a very low count. The alcohol-glycerol rennets had superior keeping qualities to the brine rennets. Chemical analysis, bacteriological analysis and graders' reports failed to distinguish the alcohol-glycerol rennet cheese from the brine rennet cheese. S.T.C.

**478. Annatto as a Cheese Colour.** M. S. CARRIE, Laboratory of the New Zealand Cooperative Rennet Co., Ltd., Eltham, New Zealand. Jour. Dairy Research 9: 72, 1938.

Standardization of annatto in aqueous solution was attacked as unsound, and it was recommended that standardization be on the basis of the depth of color produced in cheese itself. A paper color standard was suggested. S.T.C.

Other abstracts of interest are: 475, 476, 480, 481, 482, 507, 508, 509, 510, 511, 513, 514, 515, 516, 517.

## CHEMISTRY

**479. A Lipase (Tributyrynase) of Cows' Milk. I. Occurrence, Method of Estimation and Relationship of Lactation Cycle.** E. C. V. MATTICK AND H. D. KAY, National Institute for Research in Dairying, University of Reading, Shinfield, Nr. Reading, England. Jour. of Dairy Research 9: 58, 1938.

Tributyrynase in milk was estimated by determining the amount of volatile acids produced in 6 hours at 37° C. (98° F.) in a reaction mixture typically consisting of 100 ml. M/10 sodium diethyl barbiturate, 0.5 ml. pure tributyrin, and 20 ml. milk.

An enzyme which hydrolyses tributyrin was found to be present in all samples of cows' milk examined. It is associated with the aqueous rather than the fatty portion of the milk. Its optimal range of activity is in the range of pH 8.2-8.7. It is rather more thermolabile than phosphatase.

Its concentration was found to vary considerably in the milk from different cows, and during the lactation cycle in milk from individual cows. It is highest in concentration in colostrum, then the concentration falls to a minimum at about 10 days, rising later to a figure intermediate between this minimum and the colostrum value. It shows no sign of increase toward the end of the lactation period. S.T.C.

**480. The Protein Distribution in Normal and Abnormal Milk.** SAMUEL J. ROWLAND, Dept. of Agric. Chemistry, University of Reading, Reading, England. Jour. Dairy Research 9: 47, 1938.

Using the method described in the previous paper the nitrogen distri-

bution in terms of casein, albumin, globulin, proteose-peptone and non-protein nitrogen was determined for a number of samples of normal milk, of milk from cows with mastitis, and of milk persistently low in solids-not-fat content.

The milk from cows with clinical mastitis was found to be low in solids-not-fat and characterized by a decreased content of casein and an increased content of albumin, globulin and proteose-peptone substances, particularly globulin. A "casein number" calculated by the formula:  $\text{casein number} = \frac{\text{percentage of casein N}}{\text{percentage of total N}} \times 100$  was suggested as a method for detecting sub-clinical mastitis. The minimum figure for normal milk was found to be 77.0. S.T.C.

- 481. The Determination of the Nitrogen Distribution in Milk.** SAMUEL J. ROWLAND, Dept. of Agric. Chemistry, University of Reading, Reading, England. *Jour. Dairy Research* 9: p. 42, 1938.

Details are given of a semi-micro Kjeldahl method for the determination of the total, casein, albumin, globulin, proteose-peptone and non protein nitrogen of milk. The method is said to be accurate, rapid and particularly suitable for the determination of the smaller nitrogen fractions. The various nitrogen fractions are separated by the procedures described in the preceding paper. S.T.C.

- 482. The Precipitation of the Proteins in Milk.** SAMUEL J. ROWLAND, Dept. of Agric. Chemistry, University of Reading, Reading, England. *Jour. of Dairy Research* 9: p. 30, 1938.

Methods for the separation of the total protein, casein, albumin, globulin and proteose-peptone substances of milk are described.

Maximum precipitation of casein from milk samples of varying casein content was effected by the addition to 10 ml. of milk of about 80 ml. of water at 40° C. (104° F.) and 1.0 ml. of 10 per cent acetic solution followed, after 10 minutes, by 1.0 ml. of N sodium acetate solution.

For the determination of total protein the most complete separation was secured by diluting 10 ml. of milk to 50 ml. with 15 per cent trichloroacetic acid at room temperature.

Separation of globulin is effected by saturation of the filtrate (neutralized to pH 6.-7.3) from the casein determination with magnesium sulfate.

Methods also are given for the precipitation of and separation of the albumin and proteose-peptone substances. S.T.C.

## DISEASE

- 483. The Hemolytic Streptococci of Milk.** J. M. SHERMAN AND C. F. NIVEN, Cornell University. *J. Infect. Dis.* 62: p. 190, Mar.-Apr., 1938.

A study was made of a number of samples of raw and pasteurized commercial milks to determine the incidence of hemolytic streptococci and the number of kinds of species present, with an attempt to differentiate harmless types from those of possible sanitary significance. Broad-zone hemolytic types were the only ones considered. Only 8.5 per cent of the pasteurized milk samples contained hemolytic streptococci, while 18 per cent of the raw samples were positive.

On the basis of serological and physiological tests, six groups or species were recognized: *Streptococcus mastitidis* (Lancefield group B), the "animal pyogenes" (Lancefield group C), *Streptococcus durans* (Lancefield group D), and two other unidentified types.

The prevailing types of hemolytic streptococci in raw milk were found to be *Streptococcus mastitidis* and the "animal pyogenes"; the most common forms in pasteurized milk were *Streptococcus durans* and *Streptococcus zymogenes*.  
W.C.F.

**484. Brucellosis in Horses.** W. S. STONE, Experiment Station, N. Y. State Veterinary College, Ithaca, N. Y. Cornell Veterinarian 28: p. 91, 1938.

The author reviews the literature and presents some data concerning brucellosis in horses. Agglutinins are found in apparently normal horses as well as those affected with fistulous withers and poll evil. Horses in contact with cattle have a higher incidence of brucellosis than those kept away from cattle or maintained in cities. Horses may be a factor in transmitting Bang's disease to cattle. There is evidence that undulant fever may be contracted from the discharge of brucella infected horses.  
J.F.

**485. Detection of Mastitis by the Bromthymol-Blue Test, Leucocyte Count, and the Microscopic Examination of Incubated Milk.** A. C. FAY, H. W. CAVE, AND F. W. ATKESON, Kansas Agricultural Experiment Station, Manhattan, Kansas. Cornell Veterinarian 28: p. 40, 1938.

Readings of the bromthymol-blue test, the leucocyte count, and the microscopic examination of incubated milk for streptococci on repeated quarter milk samples of 114 cows are compared.

Long-chained streptococci were found in the incubated milk of 13 per cent of heifers in their first lactation period, 38 per cent of cows in their second lactation period and in increasingly high percentages in the groups in the later lactation periods.  
J.F.

**486. Age as a Factor in Susceptibility to Johne's Disease.** WILLIAM A. HAGAN, Department of Pathology and Bacteriology, N. Y. State Veterinary College, Ithaca, N. Y. Cornell Veterinarian 28: p. 34, 1938.

This paper presents evidence to show that young calves are most susceptible to Johne's disease and that natural infections occur very early in life. No cases were developed naturally in the experimental herd by animals which were exposed after the fourth month of life. Even in artificial infection, using much larger doses than animals could possibly acquire naturally, no animal more than two years of age was successfully infected. The period of incubation in dosed cases runs from nine months to more than two years. Allergic tests show that many animals that have contracted the infection are able to throw it off, usually without showing any clinical evidence of the disease. J.F.

487. Bovine Mastitis. III. A Comparison of the Bacteriological and Physiological Reactions of Normal and Mastitis Milk from Young Cows. RALPH B. LITTLE, Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, N. J. Cornell Veterinarian 28: p. 23, 1938.

Examinations of the foremilk of 8 first calf heifers before and after inoculation of the quarters with a double zone hemolytic streptococcus are compared. Plating of the milk in blood agar and the direct leucocyte count were found more efficient in detecting artificial infection than the hydrogen ion or chloride tests. J.F.

## FOOD VALUE OF DAIRY PRODUCTS

488. Milk Important Source of a New Vitamin. ANONYMOUS. Milk Dealer 27: 10, p. 67, July, 1938.

According to a recent study of 73 South Carolina families, pellagra did not occur in families which used on the average two and one-fourth cups of milk per person per day, plus fruits, vegetables, and lean meat. Another study of 29 Florida families revealed that the important difference in the diets of pellagrous and non-pellagrous families was in the amount of milk consumed. C.J.B.

489. The Effect of Commercial Sterilization on the Nutritive Value of Milk. S. K. KON AND K. M. HENRY, National Institute for Research in Dairying, University of Reading, Shinfield, Nr. Reading, England, with the collaboration of E. W. IKIN, National Institute for Research in Dairying, University of Reading, A. E. GILLAM, University of Manchester, Manchester, England, and P. WHITE, University of Reading, Reading, England. Jour. of Dairy Research 9: pp. 1-29, 1938.

### I. Introduction. S. K. Kon and K. M. Henry.

For use in experiments to determine the effect of sterilization on the

nutritive value of milk, raw milk and commercially sterilized milk taken from the same original batch of milk were secured daily from a large processing plant. For sterilization the milk, after homogenization at 4000 pounds pressure, was heated in bottles to 230° F. and held for some time at this temperature before air cooling.

**IIa. *Biological Value and Digestibility of the Proteins (Nitrogen) of Milk.* K. M. Henry and S. K. Kon.**

Paired feeding experiments with rats indicated for raw milk a biological value of 84.3 and a true digestibility of 96.4, while for sterilized milk the figures were 79.1 and 95.3 respectively. It is suggested that the lowered biological value of sterilized milk may be due to partial destruction of cystine or methionine.

**IIb. *A Note on the Effect of the Method of Feeding Dried Skimmed Milk on the Biological Value of Its Proteins.* K. M. Henry, E. W. Ikin and S. K. Kon.**

No significant difference was found in the biological value of the proteins when spray dried skimmed milk was fed separately from or mixed with a basal "nitrogen free" diet.

**III. *Effect on the Vitamin A and Carotene Content of Milk.* A. E. Gillam, K. M. Henry, S. K. Kon, and P. White.**

Butterfat was obtained by ether extraction from eleven samples of raw milk and from eleven corresponding samples of sterilized milk. The vitamin A and carotene content was estimated by colorimetric (Levibond tintometer) and by spectrophotometric tests. The results showed that the effect of the heat treatment was negligible, neither method demonstrating a loss in vitamin A, while for carotene only the colorimetric method indicated a loss of about 2 per cent.

**IV. *Effect on the Vitamin B Complex, on Vitamin B<sub>1</sub> and on Vitamin B<sub>2</sub> (Flavin) of Milk.* K. M. Henry and S. K. Kon.**

Based on the rate of growth of rats receiving various quantities of raw and commercially sterilized milk in addition to a diet deficient in vitamin B<sub>1</sub> it was calculated that about one-third of the vitamin B<sub>1</sub> originally present in milk was destroyed by sterilization. Sterilization appeared to have no adverse effect on vitamin B<sub>2</sub> (flavin). S.T.C.

## ICE CREAM

**490. Factors Influencing the Body and Texture of Ice Cream.** W. H. E. REED, Dairy Dept., Univ. of Missouri, Columbia, Mo. *Ice Cream Trade J.* 34: 5, p. 20, May, 1938.

The effect of various ingredients used in ice cream and the methods used in processing the mix on the body and texture of ice cream are discussed.

W.H.M.

- 491. Make Merchandise Men.** MALCOLM PARKS. *Ice Cream Trade J.* 34: 5, p. 16, May, 1938.

The author states, "The ice cream business today is in a state of transition, retail trends are changing, new competitive threats have arisen and the emphasis is on service and co-operative effort by the dealer and company in meeting the conditions which have arisen. The dealer looks to his company to supply him with help, encouragement and ideas to combat the inroads of competition and conditions which he is unable to cope with alone. Companies are falling down on this job, largely because sheer apathy has conspired to maintain the old status quo." A Merchandising Counselors Data Chart to be used by sales managers in obtaining data on dealers to be used as a guide in their sales promotional work is presented. W.H.M.

- 492. Who Sells Your Ice Cream for You?** IRVING B. WEBER, Sidwell Dairy Company, Iowa City, Iowa. *Ice Cream Trade J.* 34: 5, p. 45, May, 1938.

The per cent of ice cream sold through different types of outlets for this manufacturer is as follows:

Average Gallonage Rankings of Different Types of Accounts	My Gallonage Rankings for the Same Types
1st—Drug Stores . . . . .	29% of total volume
2nd—Eating Establishments . . . . .	50% of total volume
3rd—Grocery Stores . . . . .	14% of total volume
4th—Filling Stations . . . . .	4% of total volume
5th—Beer Parlors . . . . .	2% of total volume
6th—Miscellaneous Businesses . . . . .	2% of total volume

In this instance the eating establishments are out-ranking the drug stores as an outlet for ice cream. W.H.M.

- 493. Shrinkage—And How to Prevent It.** W. B. COMBS, Dairy Division, Univ. of Minn., St. Paul, Minn. *Ice Cream Trade J.* 34: 4, p. 13, April, 1938.

Factors discussed which have an effect on shrinkage in ice cream are: overrun, storage and serving temperature, composition of the mix, and size of air cells as influenced by rate of freezing and hardening. Shrinkage can be reduced in factory filled package ice cream by filling the package as quickly as possible after the ice cream comes from the freezer, placing the package immediately in the hardening room, and storing the package at a low uniform temperature. W.H.M.

- 494. How to Make Strawberry Ice Cream.** P. H. TRACY, Dairy Dept., Univ. of Illinois, Urbana, Ill. *Ice Cream Trade J.* 34: 3, p. 17, March, 1938.

Good strawberry ice cream depends on (a) a good mix, (b) the proper selection of flavoring materials, (c) the combining of the fruit with the ice cream in the proper manner, and (d) correct freezing of the ice cream.

A fresh mix free from copper contamination should be used. Only enough color to give a pinkish shade and 15 to 20 per cent of a 2.5 to 1 pack of strawberries are suggested for good results. It is important to avoid diluting the mix with fruit to a point below the legal fat standard. By adding the fruit after the ice cream is drawn from the freezer the identity of the fruit particles can be maintained. Strawberry ice cream should be sold while fresh in order to minimize the development of a stale flavor in the product.

W.H.M.

**495. Frozen Pack Fruits as Flavors for Ice Cream.** M. A. JOSLYN AND W. C. COLE, Dairy Division, Univ. of California, Davis, Calif. *Ice Cream Trade J.* 34: 3, p. 16, March, 1938.

The authors state that frozen pack fruits generally prove as satisfactory as fresh fruits for flavoring ice cream. Fruit with a pronounced flavor, and not too high in tannic acid should be used. A 2 to 1 or 3 to 1 ratio of fruit to sugar is recommended for preserving the frozen fruit for ice cream. Fifteen to 20 per cent of fruit by weight should be used in the ice cream. It is desirable to have the fruit completely defrosted before use, and fruit in smaller size is preferable to the larger size particles. Discoloration of the fruit before use tends to injure the fruit's appearance and flavor, and a high fat content mix will tend to mask the fruit flavor.

W.H.M.

**496. The Stabilization of Ice Cream with Sodium Alginate.** V. C. STEBNITZ AND H. H. SOMMER, Dept. of Dairy Husbandry, Univ. of Wisconsin, Madison. *Ice Cream Trade J.* 34: 3, p. 14, March, 1938.

Sodium alginate, "Dariloid," was subjected to various tests and compared with gelatin as a stabilizer for ice cream. Two and one-half gallon experimental mixes were made and frozen in a 10 quart vertical brine freezer. The composition of the mixes used was sugar 16 per cent, fat 13 per cent, and serum solids 10 per cent. The method recommended for adding sodium alginate was to mix it in a little cold water, and add it to the mix at 160° F.

The authors summarized their results as follows: Ice cream mix, stabilized with sodium alginate ("Dariloid") was found to possess the following properties:

1. A higher pH and lower titratable acidity than an unstabilized mix. Three-tenths per cent "Dariloid" caused an average increase of 0.15 in the pH and a decrease in the titratable acidity of 0.015 per cent.
2. Slightly more color than gelatin mixes.
3. A uniform viscosity during aging. The viscosity of the freshly made mix was practically the same as the aged mix.

4. No tendency toward wheying-off.  
 5. No stabilizer sediment when the sodium alginate had been properly dissolved.

6. A maximum whipping ability immediately after being homogenized and cooled to 40° F.

Ice cream made from mix stabilized with sodium alginate ("Dariloid") was found to possess the following properties:

1. A color slightly lighter than gelatin ice cream.  
 2. Body and texture comparable to gelatin ice cream.  
 3. A "cleaner" flavor than gelatin ice cream.  
 4. Good water-holding capacity and resistance to the effects of heat shocking.

5. No tendency toward shrinkage.

6. Smooth, clean, melt-down appearance.

W.H.M.

**497. Package Ice Cream.** KEN FORREST, Merchandising Editor, Ice Cream Field 32 (6) : pp. 7, 8, 10, June, 1938.

The author claims that unless ice cream manufacturers effectively merchandize packages and specialties, the home made dessert manufacturer "will take not only the business you hope to get, but a whole lot of business you already have."

He cites statistics from the International Association of Ice Cream Manufacturers that over 40% of the total volume sold was packages, cups and novelties.

One manufacturer is cited as successfully merchandising factory filled packages averaging only 69% overrun, in competition with cheaper merchandise.

According to the author "Your real competitor is not only your fellow ice cream manufacturer. It is the entire dessert industry, an industry that is using the power of consumer advertising to the tune of several million a year."

He emphasizes the importance of not only helping the dealer, but of using to advantage the advertising possibilities of factory filled packages.

W.C.C.

**498. Liquid Sugar.** L. F. DARDONE, Production Manager, B. W. Dyer & Co., Ice Cream Field 32 (6) : p. 43, June, 1938.

The author states that economy in price and convenience are sufficient to justify ice cream manufacturers to use liquid sugar where available. Early supplies of liquid sugar were not well controlled technically hence their composition was not uniform or satisfactory. He states "Today it is possible to obtain liquid sugar, the solid content of which is the same composition as good granulated sugar and also with practically any invert content you may require up to completely inverted sugar." W.C.C.

**499. Stale Flavor Control.** K. G. WECKEL, Dairy Industry Dept., Univ. of Wisconsin. Ice Cream Field 32 (6) : pp. 13, 14, 17, 36, June, 1938.

The author discusses in a very general manner some of the common problems encountered with stale flavors in ice cream. He stresses the need of controlling the quality of ingredients used and suggests the use of certain anti-oxidants (avenex and a trypsin preparation). W.C.C.

Other abstracts of interest are: 479, 480, 488, 489, 507, 508, 509, 510, 511, 513, 514 and 516.

## MILK

**500. This Problem of Overlapping Ordinances.** J. W. YATES, General Laboratories, Philadelphia, Pa. *Milk Dealer* 27: 9, pp. 98-101, June, 1938.

A discussion of the widespread non-uniformity in milk regulations. Examples of this non-uniformity are given. The author states that due to this non-uniformity much good milk is being rejected and does not have a free market, while other milk of inferior quality, in some instances, is permitted to be sold. C.J.B.

**501. Soft Curd Milk.** ANONYMOUS. *Milk Dealer* 27: 9, pp. 62-66, June, 1938.

A review of the research work which has been done by the dairy schools on soft-curd milk. C.J.B.

**502. Homogenized Milk.** ANONYMOUS. *Milk Dealer* 27: 9, pp. 37, 58, June, 1938.

Further reports on what dealers who distribute homogenized milk think of the product. These dealers report a steady increase in homogenized milk sales: Some of them also report increased sales of cream due to the sale of homogenized milk. C.J.B.

**503. Detecting Under Pasteurization by Means of the Phosphatase Test.** DR. T. H. BUTTERWORTH, Dept. of Health, San Antonio, Tex. *Milk Dealer* 27: 9, pp. 33, 66-70, June, 1938.

A brief description of the phosphatase test and how it is of value to the milk-plant operator as well as to the control official. C.J.B.

**504. Da-Lite Dairy.** ANONYMOUS. *Milk Dealer* 27: 8, pp. 46, 47, 67, May, 1938.

A brief description of the Holbrook Farms Dairy at Brentwood, Maryland. C.J.B.

**505. Efficiency and Sanitation.** ANONYMOUS. *Milk Dealer* 27: 8, pp. 44, 45, 82, May, 1938.

A description of Moore Brothers' Dairy at Ames, Iowa. C.J.B.

- 506. Homogenized Milk.** ANONYMOUS. *Milk Dealer* 27: 8, pp. 38, 39, 69, May, 1938.

The usual response to a questionnaire sent to milk distributors from Virginia to the State of Washington, and from Canada to Tennessee, was to the effect that "once customers use homogenized milk they like it very much and don't change back to regular milk," or from month to month its popularity to date has been increased.

The type of outlet of homogenized milk and the reaction of individual distributors to the product are also given. C.J.B.

*Editor's Note:*—This article is based on the response of 15 milk distributors who have been distributing homogenized milk from 1 month to 10 years. These distributors were contacted by The Milk Dealer to learn the favor accorded this product.

Other abstracts of interest are: 474, 475, 476, 479, 480, 481, 482, 483, 485, 487, 488, 489, 507, 508, 509, 510, 511, 512, 513, 514 and 516.

### MISCELLANEOUS

- 507. Minimum Wage Legislation.** BERNARD SUMMER, *Ice Cream Trade J.* 34: 4, p. 24, April, 1938.

The author presents the fourth of a series of tables showing violations and penalties, by states, as outlined in the Minimum Wage Legislation. W.H.M.

- 508. Showmanship in Business.** ZENN KAUFMAN. *Ice Cream Field* 32 (6): p. 23, June, 1938.

This is a concluding article of a series by the author. He states "Keep your advertising simple by emphasizing one basic appeal possessed by your product. You may want to stress purity, economy, flavor, healthfulness, or reputation. All these factors have their merits but when thrown on the public all in a heap their effectiveness is lost." W.C.C.

- 509. Social Security.** J. S. SEIDMAN, C.P.A., Director, New York Chapter, National Association of Cost Accountants. *Milk Dealer* 27: 10, p. 60, July, 1938.

A brief discussion of the link between payroll taxes and income taxes formed by a recent decision which allows employers to deduct payroll taxes on their Federal income tax returns. C.J.B.

- 510. Microbe "Death Ray" Lamp.** ANONYMOUS. *Milk Dealer* 27: 10, pp. 36-37, July, 1938.

An ultra-violet lamp, developed by Drs. Rentschler and James, is reported to give 99.99 per cent sterilization in a few seconds time. C.J.B.

- 511. A New Light Weight Stainless Steel.** ANONYMOUS. Milk Dealer 27: 10, p. 33, July, 1938.

Ludlite, a new stainless steel, consists of a Silcrome stainless steel facing approximately 0.0095 inch thick, with a tough, waterproof, flexible backing of non-metallic material. The Silcrome stainless steel and the flexible backing are permanently combined under heat and pressure.

Ludlite has already been used as a building material in many ways. Its flexibility and relatively low cost opens almost unlimited possibilities for use in the bottled milk industry. C.J.B.

- 512. A Cornhusker Sees European Dairying.** PROF. H. P. DAVIS, Dairy Husbandry Department, Univ. of Nebr. Milk Dealer 27: 9, pp. 76-79, June, 1938.

The author describes the dairy industry of Europe. C.J.B.

- 513. Highlights of the New Tax Law.** J. L. SEIDMAN, C.P.A., Director, New York Chapter, National Association of Cost Accountants. Milk Dealer 27: 9, pp. 42, 66-67, June, 1938.

The author discusses the effect of the new tax law on corporations and individuals. Income tax rates, capital gains and losses, and other provisions of the new law are discussed. C.J.B.

- 514. Some Practical Suggestions on Fighting Flies.** ANONYMOUS. Milk Dealer 27: 8, pp. 74-76, May, 1938.

Practical method of preventing the access of flies into the milk plant and methods of disposing of those which get into the plant are given. C.J.B.

- 515. Lanital, the New Textile Material Made from Casein.** ANONYMOUS. Milk Dealer 27: 8, p. 72, May, 1938.

A brief description of the process and present status of obtaining a spinning fibre from casein. C.J.B.

- 516. The Need for Scientific Cleaning and Sterilization of Dairy Equipment.** W. E. NOYES, The Diversey Corporation, Chicago, Ill. Milk Dealer 27: 8, pp. 52-60, May, 1938.

The author gives a full discussion of the following points:

1. The fundamental factors to consider in analyzing the cleaning job.
2. The factors to consider in selecting a cleaning compound.

3. Properties which a cleaner for general use in the plant should have.
4. Points to check in the operation of the can washer.
5. Properties to consider in selecting a washing product to use in the bottle washer.
6. Properties to consider in selecting a sterilizer for use on the dairy farm and in the milk plant.
7. Methods of using chemical sterilizers. C.J.B.

**517. Preparation of Cultures.** JOSEPH BURNS, Mgr., Capitol Dairy, Madison, Wis. *Milk Dealer* 27: 8, pp. 49, 78, May, 1938.

A description of the preparation and control of cultures. The author gives the following 4 laws of culture control:

1. Use good mother culture.
2. Sterilize all utensils and containers before and during process of manufacture.
3. Use clean base for medium. The skim or whole milk used must be fresh.
4. Have proper and uniform incubating temperature, together with correct length of time. C.J.B.

## PHYSIOLOGY

**518. Intervals in the Electrocardiograms of Calves Fed Cod Liver Oil.** LEROY L. BARNES, GEORGE K. DAVIS, AND C. M. McCAY, Laboratory of Animal Nutrition, Cornell University, Ithaca, N. Y. *Cornell Veterinarian* 28: p. 16, 1938.

The feeding of various levels of cod liver oil did not affect the intervals in the electrocardiograms of calves. There was a gradual increase in the intervals, most prominent in the P.R. intervals, as the animals grew older. Histological studies of the hearts revealed no lesions. J.F.

# JOURNAL OF DAIRY SCIENCE

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## ABSTRACTS OF LITERATURE

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## ABSTRACTS OF LITERATURE

### BACTERIOLOGY

19. **A Study of Comparative Methods and Media Used in Microscopical Examination of Creamery Butter. I. Yeast and Mold Counts.** G. W. SHADWICK, Beatrice Creamery Co., Chicago, Ill. *Food Research* 3, 3, 287, May-June, 1938.

This paper presents the results of a comparative study of yeast and mold counts for salted and unsalted butters using as culture media freshly prepared potato-dextrose agar and the dehydrated potato-dextrose, malt, peptonized-milk, whey, and wort agars prepared by the Difco Laboratories.

F.J.D.

520. **Heat Resistance Studied on Spores of Putrefactive Anaerobes in Relation to Determinations of Safe Processes for Canned Foods.** C. T. TOWNSEND AND J. R. ESTY, Univ. of Calif., Berkeley, Calif., AND F. C. BASELT, American Can Co., New York City. *Food Research*, 3, 3, 323, May-June, 1938.

Thermal death curves for *Cl. botulinum* in neutral phosphate media and in vegetables and milk are different. Data are presented from which safe temperatures and time intervals for sterilization may be chosen.

F.J.D.

521. **The Respiration of the Rod-Shaped Lactic Acid Bacteria.** P. ARNE HANSEN, Royal Tech. College, Copenhagen. *Zentr. Bakt.* II, 98, 289-297, 1938.

The rate of respiration of 12 strains of lactic acid bacteria of known origin has been studied. Species of the genera *Thermobacterium* and *Streptobacterium* show very low rate of respiration and are not inhibited by hydrocyanic acid, while the genus *Microbacterium* gives high values and the rate of respiration is considerably decreased by HCN. It is recommended to consult quantitative respiration experiments in taxonomic work.

J.C.M.

522. **Counting Bacteria.** S. ORLA-JENSEN AND G. FAULENBORG, Royal Tech. College, Copenhagen. *Zentr. Bakt.* II, 97, 387-389, 1938.

Comparative counts of bacteria were carried out on milk samples each of which were divided in four parts and treated as follows: one was left raw; the second pasteurized at 63° C for 30 min.; the third heated at 80° C for 2 min.; the fourth sterilized. Both plate counts and direct microscopic

counts were used. In the latter case the individual cells as well as groups of cells were separately recorded. Observations were carried out over a period of 10 days, samples being removed at once and at intervals, temperature of incubation was 5° C., chloroform was added. The dead bacteria present gradually disappeared, after two days in the sterilized milk; after 8 days in the milk heated to 80° C. The simultaneous use of plate and direct counts is advocated.

J.C.M.

**523. The Hemolytic Streptococci of Milk.** C. F. NIVEN, Dept. of Dairy Industry, Cornell Univ., Ithaca, N. Y. *Milk Dealer*, 27, 11, 64-67, August, 1938.

A total of 313 samples of commercial milk, 68 raw and 245 pasteurized, were examined for hemolytic streptococci. Narrow-zone hemolytic types in blood agar, the most typical form of *Streptococcus mastitidis*, were not considered. Only 8.5 per cent of the pasteurized samples contained hemolytic streptococci, as here defined, whereas broad-zone hemolytic types were obtained from 18 per cent of the raw samples.

The cultures isolated were studied serologically and physiologically, and, on the basis of these results, six groups or species were recognized: *Streptococcus mastitidis* (Lancefield group B), the "animal pyogenes" (Lancefield group C), *Streptococcus durans* (Lancefield group D), *Streptococcus zymogenes* (Lancefield group D), and two other types which differ serologically and physiologically from any adequately described species of streptococcus.

The prevailing types of hemolytic streptococci in raw milk are *Streptococcus mastitidis* and the "animal pyogenes"; the most common forms in pasteurized milk are *Streptococcus durans* and *Streptococcus zymogenes*.

C.J.B.

**524. The Phenomenon of the Extensibility of Ferments.** W. M. BOGDANOW, Institute for Scientific Research in the Dairy Industry, Leningrad, U.S.S.R. *Le Lait* 18, 176, 576-582 (June, 1938).

An organism is described which causes the development of a stringy condition in milk. A stable symbiosis possesses high proteolytic activity. During prolonged incubation of the ferment, the acidity gradually decreases and a strong peptonization of the albumin takes place. The combination of organisms described is acceptable for cheese making.

A.H.J.

## BREEDING

**525. A Factor in Breeding Efficiency of Dairy Cattle.** HOWARD CLAPP. *Proc. Amer. Soc. Animal Prod.* 30, 259-265, 1937.

At this large breeding establishment (Pabst Farm) it was found that the mean interval between calving and the first oestrus following was 46.4

days for cows milked twice daily, 69.4 days for cows milked four times daily (test cows), and 71.8 days for cows nursing calves. Among the group on test there was no difference in interval due to the month of freshening, but in the group not on test there was a slight significant difference, the shortest intervals occurring in December to February freshening cows. G.C.W.

Other abstracts of interest are 542, 554, and 560.

## BUTTER

- 526. The Spreading Capacity of Butter.** G. W. SCOTT BLAIR, National Institute for Research in Dairying, Univ. of Reading, Shinfield, Nr. Reading, England. *Jour. of Dairy Research* 9, 208-214, 1938.

An instrument designed to study the rheological properties of butter is described. The time required for a given weight to compress a cylinder of butter through a given distance is used to calculate the viscosity of the butter. S.T.C.

- 527. Studies of the Keeping Quality of Butter in Cold Storage.** O. R. OVERMAN, O. F. GARRETT, AND H. A. RUEHE. *Ill. Agric. Exper. Sta. Bull.* 446, Sept., 1938.

"The purpose of this investigation was to study butters of differing quality and from differently treated creams during extended periods of storage at customary storage temperatures in order to determine the influence of various factors upon the keeping quality of the butter, and especially to determine whether correlation exists between the keeping quality of butter and the results obtained by a laboratory examination of the fat."

A review of the literature and bibliography of sixty-six references is given.

During the investigation reported the behavior in cold storage of thirty-six different butters ranging in quality from poor to excellent was studied. The butters were stored in 3 pound packages, 10 to 12 packages of each butter being available. The butters were subjected to laboratory examination at definite intervals.

The examination included scoring of the butter and the determinations on the filtered fat of index of refraction, iodine absorption number, saponification number, soluble acids, insoluble acids, free-fatty acids, acetyl value, Reichert-Meissl, Polenske, and Jensen-Kirschner numbers, the pH of the serum and the computation of the mean molecular weights of the fats. The rates of oxygen absorption of the fats were determined also.

The following observations and conclusions were made as a result of this study:

1. The judge's score of a butter, although a statement of quality at the time of scoring does not give any indication of its keeping quality.

2. The particular chemical and physical constants determined do not show correlation with keeping quality.

3. The induction periods and the rates of oxidation vary so irregularly that there is no evidence of any relation of these to keeping quality.

4. Butter made from sweet cream scored higher and held its score better in storage than butter made from the same cream after ripening with starter and partial neutralization, and the latter was correspondingly better than butter made from the same cream after spontaneous souring and neutralization to the same percentage of acidity.

5. Different neutralizers used to reduce the acidity of separate lots of the same cream to approximately the same percentage of acidity did not affect the initial score or the keeping in storage.

6. Butter from cream ripened with starter to .44 per cent acidity and churned without neutralization scored higher and held its score better in storage than did butter from the same cream after it was ripened with starter to .61 per cent acidity and churned without neutralization. The latter was correspondingly better than butter churned from the same cream without neutralization after ripening with starter to .82 per cent acidity.

7. Overneutralization of cream produced a butter with low initial score and poor keeping quality.

8. Butter churned from sweet cream and salted scored slightly higher but lost score more rapidly than unsalted butter from the same cream.

9. The butters which were given the highest initial scores and held their scores best of all those studied in these investigations were churned from fresh sweet cream separated from fresh sweet whole milk. These butters were not salted and were of salable quality after about two years in storage.

O.R.O.

## 528. Treatment and Transformation of Milk, Improvement of Quality.

The Aroma of Butter. W. MOHR, Kiel, Germany. *Le Lait* 18, 177, 743-758 (July, August, 1938).

The principle aromatic substance in butter is diacetyl. The formation of diacetyl is due to the action of bacteria on citric acid. Formation of diacetyl and acetyl methyl carbinol are related but the mechanism of their formation is not definitely known. Among the problems in this connection are whether both substances are formed simultaneously or whether diacetyl is formed from the oxidation of acetyl methyl carbinol and if such is the case, by what mechanism, or if diacetyl is produced simply from acetaldehyde in the course of oxidation with subsequent condensation. Increasing acidity and aeration favor the production of diacetyl. High temperatures at the time of proper acidity, notably the temperature optimum for the growth of bacteria are not favorable for the production of

diacetyl. Diacetyl is formed more rapidly between 12° C. (53.6° F.) and 16° C. (60.8° F.) or between 17° C. (62.6° F.) and 21° C. (67.8° F.) with cooling of the culture to 10° C. (50° F.) when the acidity has increased to 23° S.H. The conditions that apply to the formation of diacetyl and acetyl methyl carbinol in cultures apply equally in the cream used for butter making. During churning the content of diacetyl and acetyl methyl carbinol increases and is significantly higher in the buttermilk than in the cream. The butter contains about 1/4 the diacetyl and 1/15 to 1/16 the acetyl methyl carbinol found in the ripened cream. The diacetyl and acetyl methyl carbinol are found distributed throughout the butter in the aqueous phase of the serum and not at all in the fatty phase. The washing of the butter eliminates a considerable part of the diacetyl and acetyl methyl carbinol. At ordinary temperature and for short storage periods, i.e., at 10° C. (50° F.) and up to 4 days, there results an increase in diacetyl. Within 10 to 12 days, however, the diacetyl decreases. On storage at low temperature 0° to 10° C. (32° F. to 14° F.) the content of diacetyl does not undergo change.

Butter made from sweet cream contains only very small amounts of diacetyl, nor is diacetyl formed during prolonged storage for as long as 6 months. Methods of determining diacetyl and acetyl methyl carbinol are discussed. It is emphasized that differences in methods and technique account for the variation in results obtained by different investigators and that this makes it difficult to interpret results from different laboratories. A rapid practical method that will give reliable results and that will be applicable both to research and control laboratories is needed.

A.H.J.

**529. Keeping Quality of Butter.** JOHANNES JENSEN AND W. RITTER, Aabenraa, Denmark, and Berne-Liebefeld, Switzerland. *Le Lait* 18, 177, 758-773 (July, August, 1938).

Fishy flavor in butter is due to the formation of tri methylamine originating from lecithin as a result of hydrolysis and oxidation. The oxidation is markedly accelerated by the presence of small amounts of metals, particularly iron and copper. Copper is about 13 times as effective as iron in catalysing the oxidation. Pasteurization at 90° C. (194° F.) of the cream from which the butter is made operates to prevent the development of the fish flavor. This is due in part to destruction of bacteria and probably partly to antioxidants formed at the high pasteurization temperature. Some types of bacteria in butter reduce the rate of fishy flavor development in butter by consuming the oxygen contained in the butter. The acidity of the cream from which the butter was churned is important in controlling the keeping quality and should not vary from the desired acidity of 23 to 25 S.H. Stored butter derived from cream of acidity 20 S.H. has a tendency to mold, while butter derived from cream of acidity more than 29

S.H. becomes rancid prematurely. Butter oil prepared by the boiling method is a satisfactory means of storing butterfat for considerable periods with less danger of off flavor developing than if butter itself is stored. The butter fat prepared by this method, however, possesses flavors imparted to it by the cooking process while the natural aroma of the original butter may have been driven off. Methods of packaging butter to prevent the development of off flavors is discussed. Light is the chief factor involved in off flavor development in packaged butter. Protection from light by black paper or metal foil or papers containing materials that absorb ultra violet are described. Variations in the chemical characteristics of butter take place from season to season as a result of change in the feed of the cows. Thus the average saponification number was found to be 232 from December to February and 222 in summer. The Reichert-Meisssl-Vollny number had a value of 33 in winter and 29 in summer. The Polenski number had an average value of 5 in winter and 3 in summer. The xylol index averaged 23 from February to April and about 20 in July and August. The index of refraction was 1.4537 in winter and increased to 1.4555 in summer. The iodine number increased from 35 in January to 41 in summer. The effects of individual feeds and feeding régimes on the chemical constants of the butterfats are discussed and data presented. A relation between the fat content of the milk and the iodine number of the fat is observed. If the fat content of the milk increases, the iodine number of the fat decreases. Butter of an iodine number of 28.5 to 34.5 is, from the physical point of view, considered to be satisfactorily conditioned. Butter with an iodine number above 34.5 is considered too soft and below 28.5 it is too hard and brittle.

A.H.J.

Other abstracts of interest are 519 and 559.

## CHEESE

530. **The Calcium and Phosphorus Contents of Some Types of British Cheese at Various Stages during Manufacture and Ripening.**  
E. C. V. MATTICK, National Institute for Research in Dairying,  
Univ. of Reading, Shinfield, Nr. Reading, England. *Jour. of Dairy Research* 9, 233-241, 1938.

The calcium and phosphorus contents at various stages in the manufacture and during ripening of Cheddar, Cheshire, Leicester, Lancashire and Stilton cheese were determined. There was little difference in the values of the hard pressed varieties, however, Stilton cheese contained much less calcium and considerably less phosphorus than the hard pressed cheese. From about 50 to 60 per cent of the original calcium of the milk was left in the hard pressed cheese after 8 months, and only about 7 per cent in Stilton.

S.T.C.

- 531. The Rôle of Bacteria in Milk Destined for the Fabrication of Gruyère or Emmental Cheese.** W. DORNER, Agronomic Engineer for the Federal Institution of the Dairy Industry, Liebefeld, Switzerland. *Le Lait* 18, 175, 449-455 (May, 1938).

The numbers of bacteria play a very important rôle in the preparation and ripening of cheese. Harmful bacteria are more harmful when they are more numerous, and useful bacteria may be harmful when they are too numerous. *Bacterium amylobacter* causes a swelling up of the cheese and *Bacterium proteolyticum* causes a secondary offensive fermentation. With the exception of *Bacterium coli* and *aerogenes* when they come from mastitis, the pathogenic bacteria are not susceptible directly of causing difficulty with the preparation of Gruyère or Emmental cheese. A.H.J.

- 532. Volatile Acids of Cheese. II. Methods of Extraction.** E. R. HISCOX, J. HARRISON, AND J. Z. WOLF, National Institute for Research in Dairying, Univ. of Reading, Shinfield, Nr. Reading, England. *Jour. of Dairy Research* 9, 227-232, 1938.

A method for estimating the volatile acids in cheese based on the steam distillation of a water extract of the cheese is described. Considerably higher results were secured with this method than by direct steam distillation. S.T.C.

- 533. Volatile Acids of Cheese. I. Retentive Power of Cheese and Its Constituents.** E. R. HISCOX AND J. HARRISON, National Institute for Research in Dairying, Univ. of Reading, Shinfield, Nr. Reading, England. *Jour. of Dairy Research* 9, 215-226, 1938.

To determine the exhaustiveness with which the various acids could be recovered by steam distillation acetic, propionic, butyric, caproic, caprylic and lauric acid solutions with added cheese fat, butter fat, cheese protein and casein were steam distilled using a standard procedure. The fat portion of the cheese was found to have a retarding effect on the distillation of the higher volatile fatty acids. The cheese protein appeared to be capable of a permanent retention of a part of some of the acids present. The results indicate that a more accurate picture is gained of the distribution of the acids present by collecting only "twice the original volume" rather than by distilling to "5 times the original volume." S.T.C.

- 534. Chemical Changes During the Melting of Natural Cheese.** M. KVERON, Polytechnic High School, Prague, Czechoslovakia. *Le Lait* 18, 176, 561-575 (June, 1938).

There is little change in the properties of the butterfat in cheese as a result of melting in the presence of disodium phosphate or sodium citrate.

No significant change results in the Reichert-Meissl, Wauters Polenske or iodine values. The acidity of the fat is reduced slightly (on the average 2.5%) due to the melting in the presence of the alkaline salts. The soluble nitrogen is much higher in the melted cheese than in the natural cheese, increasing from about 11% of the dry matter in the natural cheese to about 40% in the melted cheese. The increase in soluble nitrogen is greater when disodium phosphate is used than when sodium citrate is used. The ash content of the melted cheese is higher than for natural cheese due to the added mineral salts. It is suggested that the higher ash content may be used to distinguish between natural and so-called "processed" cheese. The melting of the cheese increases the titrable acidity due to the effects of the alkaline salts on the proteins of the cheese. A.H.J.

**535. Milk Organisms in Cheese Making.** CONSTANTINO GORINI, Milan, Italy. *Le Lait* 18, 177, 711-712 (July, August, 1938).

It is emphasized that milk organisms play a favorable role in the ripening of Gruyere and Emmental cheese because of their solubilizing action on casein. This group of organisms accordingly acts as activators for the lactic ferments. Gorini states that Dorner was incorrect in his conclusion that Gruyere and Emmental were not favorably affected by this group of organisms just as was the case for the other types of cheese. Even though organisms themselves might be destroyed in the process of cheese making, enzymes elaborated by them exert a favorable role in the maturing of cheese. A.H.J.

## CHEMISTRY

**536. The Determination of the pH of Lactic Acid Casein.** JEAN PIEN AND M. WEISSMANN, Laboratories of the Farmers Union, Paris, France. *Le Lait* 18, 175, 455-462 (May, 1938).

The pH of the casein solution may be used to indicate if the protein has been precipitated above its iso-electric point, if the washing has been incomplete and the casein thus contain casein lactates, and if the precipitation has taken place at a pH lower than 4.7. The solution is prepared for the determination of pH in the following manner: into a 100 cc. flask equipped with a ground glass stopper are added 50 cc. of twice distilled boiled water and 5 grams of casein. After shaking, the suspension was allowed to stand for 30 minutes if the casein was finely ground and for two hours if it was coarsely ground. The two hour period may be adopted for all caseins with shaking from time to time, if the flask is tightly stoppered. Toward the end of the soaking period, the flask is allowed to stand and the supernatant liquid decanted into the electrode vessel. The hydrogen electrode required a longer period to attain equilibrium than the quinhydrone or the antimony

electrode in which cases equilibrium was attained immediately. Equivalent results were obtained with all these electrodes as well as with brom cresol green indicator. A.H.J.

- 537. Relationship of the Density in Dairy Products.** JEAN PIEN AND G. MAURICE, Laboratories of the Farmer's Union, Paris, France. *Le Lait* 18, 176, 582-610 (June, 1938).

Equations are developed from which the density of various dairy products can be calculated from a knowledge of the density of fat and solids-not-fat in the dairy product. A.H.J.

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

- 538. The Progress in Italy in the Fabrication of Synthetic Fibres Derived from Casein.** G. GENIN, Engineer E.P.C., Paris, France. *Le Lait* 18, 175, 481-484 (May, 1938).

Caseins originating from different sources are blended in proportions to give a uniform starting material. Water and other substances are then added and a viscous paste is formed. The fibres are formed by forcing the paste through holes 0.02 to 0.03 mm. in diameter. The casein fibres are coagulated and hardened by passing them through warm sulphuric acid. The acid in the fibres is then neutralized by immersion in an alkaline bath after which the fibres are cut into small bits which form the "flocks." The small branchlets are then immersed in a formaldehyde bath in which they remain for 10 to 15 hours. After washing and drying, the product is ready for spinning and weaving. The chief difference between the chemical composition of natural and synthetic wool is the sulphur content. The casein wool has a much lower sulphur content. It consequently is a superior insulating material than natural wool. Synthetic wool fibres when boiled with water for 3 hours did not lose weight. When boiled in an alkaline soap solution there was a small loss in weight, but less than that lost by treating genuine merino wool in the same way. Fibres are also made with mixtures of casein and cellulose (viscose used in the manufacture of rayon) materials. It is suggested that such products be called "Serin-laine" when the percentage of casein is greater than the percentage of cellulose and "Rayonlaine" when the percentage of casein is lower than that of cellulose. The nitrogen content indicates the proportion of cellulose and casein in such products. A.H.J.

Other abstracts of interest are 536, 558 and 559.

## DISEASE

- 539. The Effect of Subclinical Mastitis on the Solids-Not-Fat Content of Milk.** S. J. ROWLAND AND M. ZEIN-EL-DINE, Department of Agri-

cultural Chemistry, Univ. of Reading, Reading, England. Jour. of Dairy Research 9, 182-184, 1938.

The solids-not-fat content (expressed as percentage of the fat free milk) was determined for 247 samples of milk from individual quarters of 62 cows. The samples were also examined bacteriologically for the presence of *Streptococcus agalactiae*. Eighty-eight per cent of the samples below 8.80 per cent solids-not-fat were from infected quarters. S.T.C.

**540. The Casein Number. A Chemical Method of Diagnosis of Mastitis.**

S. J. ROWLAND AND M. ZEIN-EL-DINE, Department of Agricultural Chemistry, Univ. of Reading, Reading, England. Jour. of Dairy Research 9, 174-181, 1938.

Additional data on the accuracy of the casein number

$$\left( \frac{\text{percentage of casein N}}{\text{percentage of total N}} \times 100 \right)$$

as a chemical method for the detection of mastitis are reported. The number was determined for 247 samples of milk from the individual quarters of 62 cows, and the samples were examined bacteriologically for *Str. agalactiae*. For the diagnosis of mastitis from the casein number, a figure of 78.0 and less was taken as indicating an infected quarter. The chemical results differed from the bacteriological for 21 out of a total of 243 quarters. The casein number was considered from this data to be a reliable diagnostic method. S.T.C.

**541. The Incidence of Mastitis in Cows Yielding Milk Low in Solids-**

**Not-Fat.** A. S. FOOT AND P. M. F. SHATTOCK, National Institute for Research in Dairying, Univ. of Reading, Shinfield, Nr. Reading, England. Jour. of Dairy Research 9, 166-173, 1938.

An average of 19.5 per cent of the animals in milk in twenty-nine herds comprising 934 cows were found to yield milk low in solids-not-fat (below 8.5 per cent) as determined by analysis of the milk from two consecutive milkings at 5 to 6 week intervals during the first three months of 1937. Of the low solids-not-fat cows which were not stale, evidence of mastitis infection was found in an average of 61.5 per cent of cases. Evidence of mastitis infection was based on physical examination, positive brom-cresol test or bacteriological examination for the presence of *Str. agalactiae*. There were apparently at least twice as many subclinical cases of mastitis as clinical cases. S.T.C.

Other abstracts of interest are 542 and 560.

## FEEDS AND FEEDING

**542. Reproduction on Rations Free from Vitamin E.** BYRON H. THOMAS

AND C. Y. CANNON, Iowa State College. *Proc. Amer. Soc. Animal Prod.* 30, 59-63, 1937.

A suitable grain mixture and alfalfa hay were depleted of Vitamin E by treatment with an ether solution of ferric chloride and fed to goats in the proportion of 2:1. Over a period of 4½ years both males and females reproduced normally. These same feeds fed in various proportions to rats invariably produced symptoms characteristic of Vitamin E deficiency in this species.  
G.C.W.

**543. Phosphorus Deficiency in Cattle as a Result of Conditions Other than Low Phosphorus Content of the Soil and the Feeding Stuff Grown Thereon.** E. B. FORBES AND S. R. JOHNSON. *Proc. Amer. Soc. Animal Prod.* 30, 340-344, 1937.

Reports on a survey of cattle suffering from phosphorus deficiency in Pennsylvania with a description of environment, symptoms, and methods employed in bringing about recovery.  
G.C.W.

**544. Lactic Silage with a Pasteurized Thermophile.** CONSTANTINO GORINI, Milan, Italy. *Le Lait* 18, 177, 673-681 (July, August, 1938).

Satisfactory silages are high in lactic acid, unsatisfactory silages are high in butyric acid. Lactic acid fermentations in silage are usually the result of proper conditions and temperature of the silage and absence of air. A more certain method of preparing satisfactory silage is to seed with a lactic acid producing organism. A Thermophile capable of producing lactic acid is recommended. In order to prepare silage with this organism, it is necessary to compress the silage uniformly and to control the temperature so that it rises to about 60° C (140° F) neither too slowly nor too rapidly. Silage prepared in this manner is eaten readily by cattle, and is green and fresh in appearance with a minimum of wilted and faded leaves. Such silage is usually high in vitamin content as it can be prepared from freshly cut material and can be taken from the fields wet with rain or dew.  
A.H.J.

**545. The Preservation of Fodders by the Addition of Acids.** JOEL AXELSSON, Upsala, Sweden. *Le Lait* 18, 172, 216-220 (February, 1938).

The use of hydrochloric and sulphuric acids or mixtures of these acids (A. I. V. process) is more satisfactory for preserving silage than organic acids. Silage preserved with these mineral acids shows lower losses in nutritive ingredients during storage, is higher in its vitamin A content, and stimulates milk production. Legumes require more acid when made into silage than do grasses. The pH of acidulated silage should be 3.5 to 4.0. The use of acid operates to soften the woody tissues of the silage and

renders them more readily digestible. The flavor of milk from cows fed acidulated silage is equal to that of summer milk and the butter is also improved. However, such milk was not satisfactory for the production of Emmenthal cheese, but was excellent for the production of sharp flavored cheeses.

A.H.J.

Other abstracts of interest are 554, 557 and 560.

## FOOD VALUE OF DAIRY PRODUCTS

- 546. Stability of Vitamin D in Irradiated Evaporated Milk.** C. H. KRIEGER AND H. T. SCOTT, Wisconsin Alumni Research Foundation, Madison, Wis. *Food Research* 3, 3, 283, May-June, 1938.

There is little or no loss in the vitamin D potency of irradiated evaporated milk stored under average conditions for periods of two to three years, no loss whatsoever being noted after one year of holding.

F.J.D.

- 547. The Effect of Commercial Sterilization on the Nutritive Value of Milk.** S. K. KON AND K. M. HENRY, National Institute for Research in Dairying, Univ. of Reading, Shinfield, Nr. Reading, England, with the collaboration of E. W. IKIN, National Institute for Research in Dairying, Univ. of Reading, A. E. GILLAM, Univ. of Manchester, Manchester, England, AND P. WHITE, Univ. of Reading, Reading, England. *Jour. of Dairy Research* 9, 185-207, 1938.

V. The Effect of Commercial Sterilization on the Nutritive Value of Milk, K. M. Henry and S. K. Kon.

Fifteen samples of raw and fifteen samples of commercially sterilized milk from the same bulk were analyzed for vitamin C by the chemical method (titration with dichlorophenol-indophenol). The raw milk contained an average of 1.83 mg./100 ml. of total (reduced and reversibly oxidized) ascorbic acid. The corresponding figure for sterilized milk was 1.03 mg./100 ml., a loss of 43 per cent of the original value.

VI. Comparison of the Total Nutritive Value of Raw and Commercially Sterilized Milks, K. M. Henry, E. W. Ikin and S. K. Kon.

Paired feeding experiments with rats indicate that the total nutritive value of commercially sterilized milk is somewhat lower than that of raw milk and that vitamin B<sub>1</sub> is the first limiting factor. Rats getting limited but equal amounts of milk in addition to a basal diet which applied only protein, energy and minerals grow better on raw than on sterilized milk. The addition of 5 per cent brewer's yeast to the basal diet corrected this deficiency.

**VII. Conclusions. S. K. Kon.**

From the nutritional aspect sterilized milk has been shown to be inferior to raw or to pasteurized milk. Sterilization decreases slightly the biological value of the proteins, destroys half the vitamin C and 30 per cent of the vitamin B<sub>1</sub>.  
S.T.C.

**548. Groups of Milk. R. DUJARRIC DELA RIVIERE AND N. KOSSOVITCH. Le Lait 18, 474-481 (May, 1938).**

Human milks contain  $\alpha$  and  $\beta$  agglutinins for human red blood cells. Human milks may also be classified into 4 groups analogous to those of blood. The presence of iso-agglutinins is less regular in mother's milk (80.9% of the cases) than in their blood serum. The percentage of cases where iso-agglutinins are present in the milk is appreciably analogous for all groups (A:83.8; O:83.1). However, the percentage of group B is slightly higher (86.3). Mother's milk derived from the blood group AB (cases where the blood serum does not possess agglutinins) do not contain agglutinins; the red globules of group O (which are poor in agglutininogen) are never agglutinated by a lacto serum. The milk of group O $\alpha\beta$  always possesses the two agglutinins  $\alpha$  and  $\beta$  more or less strongly. Agglutinin  $\alpha$  in general acts more strongly than agglutinin  $\beta$ . Divergence between the agglutinins of blood serum and the agglutinins of milk serum of the same mother had not been found. Relation between anaphylactic shock and "group" of the milk ingested or between tolerance for milk and the group of the milk ingested have not been worked out. However in 31 cases where nutritional difficulties were being experienced with milk, it was found that the blood groups of the infant were different from the milk group of the nurse.  
A.H.J.

**549. New Aspects of Lactic Therapy. JEAN PIEN, Chemical Engineer, Director of the Laboratory of the Farmers Union, Paris, France. Le Lait 18, 177, 699-711 (July, August, 1938).**

Biochemical and clinical manifestations of intestinal intoxication due to harmful intestinal flora are reviewed. Milk organisms by which such situations may be corrected are discussed. Lactic therapy demands that ferments be present which can grow in the intestine, that only living organisms of strong virulence be employed, and that the numbers of living organisms in the cultures used be high.  
A.H.J.

**550. A Physico-chemical Theory of Artificial Infant Feeding. L. PIKLER, Budapest, Hungary. Le Lait 18, 177, 681-698 (July, August, 1938).**

The author discusses the changes in colloidal and ionic conditions of cows milk that takes place as a result of the various modifications that are

accorded it in making it satisfactory for infants. Among the various treatments of milk discussed are boiling, skimming, dilution with water, addition of acids, addition of alkali, addition of salts particularly calcium salts and citrates, addition of sugar, saccharose, dextrinized flour, malt, and boiled gruel of barley, rice and oats, and the addition of dried protein, of gelatin and of gums. The effects on milk of various enzymes such as rennin, lactase, pepsin, other proteolytic enzymes, and milk lipase and stomach lipase are also discussed. The effects of these additions on osmotic concentrations and properties of the proteins, particularly of the casein, are emphasized. One hundred twelve literature citations are given.

A.H.J.

## ICE CREAM

### 551. Ice Cream Sales Index. An Analysis of Ice Cream Sales in 1938.

ANONYMOUS. Special Bulletin of the Inter. Assn. of Ice Cream Mfrs., 1105 Barr Bldg., Washington, D. C., August, 1938.

Sales of ice cream for the first four months of 1938 are compared with the same four months of 1937. For the United States, the first four months of 1938 showed increased sales. In Canada the increase for the four-month period over that of the first four months of 1937 was 6.43 per cent.

The completed survey of ice cream sales for the full year of 1937 shows the gallonage to be the greatest of any year in the history of the industry.

M.J.M.

## MILK

### 552. The Suitability of the Cremometric and the Phosphatase Tests in the Supervision of Holder Pasteurized Milk. JOHANNE E.

JACOBSEN, Royal Tech. College, Copenhagen. *Zeitschr. Untersuchung der Lebensmittel* 71, 515-521, 1936.

Both reactions can be used to indicate if milk has been heated to 62-63° C for half an hour. The phosphatase test has certain advantages over the cremometric test: (1) Homogenization has no influence on the results; (2) it can be used also on cream and skimmilk; (3) a small admixture of raw milk to pasteurized milk can be detected. However, in certain respects the cremometric test is to be preferred: (1) It does not use expensive and poisonous chemicals; (2) it is so easy to carry out that any dairy can use it; (3) finally it shows if the milk has been overheated.

J.C.M.

Author's Note: The cremometric method is not used in the United States as it depends upon cream layer reduction at temperatures higher than those required in the United States. In Denmark milk is generally heated to 62° C (145.4° F.) or higher.

- 553. How Can the Cities Be Supplied with Good and Cheap Milk.** S. ORLA-JENSEN, Royal Tech. College, Copenhagen. *Maelkeritidende* 50, 157-165, 1937.

The author advocates the double safeguard offered by compulsory pasteurization, *e.g.*, 63° C., 30 min. and vigorous veterinary control of all market milk; also cream should be pasteurized. Specially to be recommended is the pasteurization in bottles, already practiced in about 400 smaller dairies in Denmark. The bottled milk should not be distributed by each individual milk company, but by a centralized distribution agency in order to cut down the cost of distribution. J.C.M.

- 554. Variations in the Protein Content of Milk During Lactation.** E. AZARME, Institute of Animal Genetics, Univ. of Edinburgh, Edinburgh, Scotland. *Jour. of Dairy Research* 9, 121-146, 1938.

The total protein nitrogen and the casein nitrogen were determined in about 380 weekly samples of milk taken from twenty-seven individual cows of different breeds and at different stages of lactation during a period of about 6 months. The albumin plus globulin nitrogen was calculated in each case by difference. It was found that the percentage of total protein nitrogen decreases very significantly from the beginning until the 4th week of lactation, and then rises slowly until the end of lactation, the rise being more pronounced towards the end. The same was true for casein nitrogen and for albumin plus globulin nitrogen, but with the latter only the decrease at the beginning and the rise at the end was sharp. S.T.C.

- 555. The Milk of the Goat under English Conditions.** FRANK KNOWLES AND J. E. WATKIN, East Anglian Institute of Agriculture, Chelmsford, England. *Jour. of Dairy Research* 9, 153-165, 1938.

An account is given of observations made for over two years on the yield and composition of the milk of the breeds of goats distributed throughout Great Britain. In all 2662 samples from 345 animals of 8 breeds were analyzed. The average analyses reported are as follows: fat, 4.50 per cent; solids-not-fat, 8.68 per cent; lactose, 4.08 per cent; total proteins, 2.90 per cent; casein, 2.47 per cent; albumin and globulin, 0.43 per cent; non-protein nitrogen, 0.44 per cent; ash, 0.79 per cent. The average milk yield of the officially recorded goats in Great Britain was shown to be about 7½ pounds per day. S.T.C.

- 556. The Milking Pail of Jens Grand.** S. ORLA-JENSEN, Royal Tech. College, Copenhagen. *Maelkeritidende* 50, 1039-1041, 1937.

The practice of milking directly down upon solid carbon dioxide to cool milk effectively has not been found to have any value. Such large quantities

of CO<sub>2</sub> are needed that the milk gets a disagreeable flavour. The bacteria present are not inhibited under these conditions, some are even favoured in their development.

The value of filling milk pails with gaseous carbonic acid is thus open to doubt. The favorable results obtained with the device of Jens Grand are probably caused by the careful sterilization and handling of the apparatus and not by the presence of CO<sub>2</sub>.  
J.C.M.

**557. Errors Involved in the Estimation of the Lactation Yield of Protein According to the Intervals between Sampling.** E. AZARME, Institute of Animal Genetics, Univ. of Edinburgh, Scotland. Jour. of Dairy Research 9, 147-152, 1938.

An attempt was made to determine the frequency of sampling necessary to estimate accurately the lactation yield of protein. For practical purposes sampling every two weeks during the first 6 weeks and the last 4 weeks of the lactation and every three weeks for the remainder was considered sufficient.  
S.T.C.

Other Abstracts of interest are 522, 523, 524, 537, 539, 541, 546, 547, 548, 550, 558, 559, 560.

## MISCELLANEOUS

**558. Problems which Remain to Be Solved in the Dairy Industry.** G. GENIN, Chemical Engineer E.P.C., Paris, France. Le Lait 18, 176, 610-614 (June, 1938).

Problems dealing with the production of fluid milk, condensed milk, ice cream, cheese, the fabrication of dairy equipment, and the disposal of dairy wastes are discussed.  
A.H.J.

**559. Treatment of Waste Water from the Dairy.** G. GENIN, Engineer E.P.C.I., Paris, France. Le Lait 18, 177, 711-714 (July, August, 1938).

Loss of milk in the wash water of the dairy averages  $\frac{1}{2}$  to 1% of the milk passing through the plant. For cheese factories and creameries the losses run from 3% to 8%. Such losses in the dairy wastes going into streams are responsible for considerable pollution. Rather than require expensive waste disposal plants, it is suggested that efforts be made to keep concentrated wastes from going into the wash water. This may often be done inexpensively by modifying existing equipment or processes and by exercising care in the saving of possible usable dairy wastes and in the cleaning of equipment.  
A.H.J.

## PHYSIOLOGY

**560. Secretion of Milk.** DWIGHT ESPE, Iowa State College, Ames, Iowa.  
Published by Collegiate Press, Inc., Ames, Iowa. Price \$3.00.

This book was designed as a text for a course in Milk Secretion in which the principal objective is the application of the fundamental training received by the student in Anatomy, Physiology, and Nutrition to the problems dealing with the normal functioning of the mammary gland.

The material covered is well organized into three parts. Part one deals principally with the anatomical aspects of the subject, part two with the physiological, and part three with the nutritional.

The subject matter is clearly presented and should be found readable by those for whom the book is intended. This work provides a much needed text and reference work for the undergraduate student in dairy production.

The author has used a wealth of reference material and a bibliography of 695 references is appended. While the book was not designed for the research worker, the generous use of the literature makes this a useful book for the library of the investigator.

The author has made generous use of illustrative and tabular material, there being forty-nine illustrations and thirty-four tables. T.S.S.

Other abstracts of interest are 539, 541, 554, and 557.



# JOURNAL OF DAIRY SCIENCE

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## ABSTRACTS OF LITERATURE

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International Association of Milk Dealers	The Royal Technical College, Copenhagen, Denmark
National Institute for Research in Dairying, Reading, England	United States Department of Agriculture
New York Association of Dairy and Milk Inspectors	

# ABSTRACTS OF LITERATURE

## BACTERIOLOGY

561. **Abstracts of Papers from the Proceedings of the Society of Agricultural Bacteriologists—England.** Abstracts submitted to the JOURNAL OF DAIRY SCIENCE by I. G. Davis. Nat. Inst. Res. Dairy., Reading, England.

**A. "The bacteriology of clotted cream"—E. L. CROSSLEY.**

The two main factors controlling bacteria in clotted cream are bacterial multiplication during cooling and the handling operations during skimming, weighing and packing. Cooling should be as rapid as possible. The flora at this stage consists of spore forming bacilli, which may produce bitterness, but which are usually overgrown by acid forming organisms which enter later and finally predominate. Cleanliness and sterility of plant are essential to the production of a good cream.

**B. "The bacteriology of spray dried milk powder"—E. L. CROSSLEY.**

Powder from good quality milk contains far fewer bacteria than that from poor quality milk although similar types are found. The number varies from thousands up to several millions per g., thermoduric cocci forming the bulk of the flora. *B. subtilis* type aerobic spore-formers are always present. Yeasts, moulds, anaerobes (*Cl. welchii*) and heat-resisting coli are sometimes present. Acid-forming types nearly always predominate. The death rate appears to depend on the types of organisms present.

**C. "The enumeration of bacteria in cheese"—J. HARRISON.**

It was found that emulsions of ripe Cheddar cheese prepared with hand emulsifiers gave plate counts nearly ten times as high as emulsions prepared by grinding with sand in a mortar. When young cheese were used, counts about 2 or 3 times as high were obtained. Different emulsifiers gave similar counts. It is essential to take several plugs if an accurate idea of the bacterial count of a cheese is to be obtained. A device for random selection of colonies from a plate is described.

**D. "A note on the estimation of volatile acids of cheese"—E. R. HISCOX AND J. HARRISON.**

Lactic acid and CO<sub>2</sub> are responsible for errors in the estimation of volatile acids in cheese. If the fat and the ether extract are used the lactic acid error is negligible. The CO<sub>2</sub> may be removed from the distillate by drawing CO<sub>2</sub>-free air through it for 20 minutes.

**E. "The incidence of milk samples yielding acid and gas in McConkey broth at 30° C."—C. D. OXLEY.**

A test of 205 samples of milk by the methylene blue test, the "presumptive coli" test at 37° and at 30° C. showed that the last was responsible for more failures than the others. This test picks out more "poor" samples than any other.

**F. "Statistical examinations of the results of a co-operative experiment on methylene blue reduction tests and bacterial plate counts"—H. BARKWORTH AND A. T. R. MATTICK.**

A comparison of the plate count and methylene blue reduction test showed that 87 per cent of samples failed or passed both tests. The correlation between plate count and reduction time was lower in winter, although here a high correlation was found with high plate count. Four laboratories testing common samples agreed on plate counts and reduction times at 37° C., but gave differences with the keeping quality test and the reduction test at 15.5° C.

**G. "Some experiments on the value of the Methylene Blue Test and the Resazurin test for the grading of milk during winter"—S. B. THOMAS.**

A comparison of the old method of carrying out the methylene blue test with the Wilson modification showed that half hourly inversion decreased the reduction time on the average by 5 hours.

The mean reduction time for samples held in the refrigerator at 40° F. overnight was 3½ hours longer than for duplicates held overnight at 60° F.

The variations in the reduction times of duplicate tubes observed by one worker increased in number and magnitude as the reduction times increased. Quintuplicate tests by one worker, taking readings every five minutes, gave a mean coefficient of variation of reduction times as low as 0.7 per cent, whereas the mean C.V. obtained by four workers using separate water baths was 6.5 per cent, and for duplicate tests at each of four laboratories 9.4 per cent. A comparison of the Resazurin test with the methylene blue test showed that the former is worthy of further investigation.

**H. "The effect of storage time and temperature on the methylene blue reduction test for milk"—M. BRAZ AND W. A. HOY.**

The importance of time and temperature of storage is emphasized. In practice wide variations in temperature may occur. A series of tests showed that storage for 12 hours at 65° F. is equivalent to 18 hours at 60° F. A temperature of 40–50° F. has a considerable retarding effect on the test, but a storage temperature of 60° F. reveals a milk in its true character.

- I. "A comparison between the methylene blue reduction test and the plate count with changing conditions of milk production and with changing conditions of storage of milk samples before testing"—A. A. NICHOLS AND C. J. JACKSON.

A study of the comparative sensitivity of the plate count and methylene blue reduction test to bad methods of production is described. The latter is more sensitive if the samples are held at 72° F. and (for dirty conditions) at room temperature. A short holding followed by refrigeration reveals very little difference. Testing immediately after milking or after storage at low temperatures showed that the plate count provided the better index.

- J. "A comparison of the results of different methods of analysis and of sampling of milk during 1937"—M. E. PIRRIE.

The present Accredited Standard was found to be more severe than the old Grade A standard on account of the holding of small samples at atmospheric temperature for 12 or 18 hours. Both plate count and methylene blue tests give similar results if samples are held under identical conditions. Icing of samples during transit and holding is recommended.

- K. "Mastitis in relation to the methylene blue reduction test"—J. G. DAVIS AND J. McCLEMONT.

Mastitis has a much more marked effect on plate count than on methylene blue reduction time. Methylene blue is toxic to *Str. agalactiae* so that it is unlikely that this organism will either proliferate or reduce the dye in the test. The slightly shorter reduction times due to mastitis may be attributed to the higher cell content and to the increased flora. Holding the samples at 4° C. or 15.5° C. did not appear to increase the differentiation between infected and non-infected samples. The methylene blue test is of no use for the detection of mastitis.

- L. "Indirect methods of mastitis diagnosis"—M. ZEIN-EL-DINE AND S. J. ROWLAND.

Clinical examination (by palpation) could only detect 7% of the cases. "Clots" or flakes of the fore-milk, and brom-thymol-blue papers could detect 12 and 14% respectively of the positive quarters. No "false positives" could be found with these three tests.

The brom-cresol-purple papers could detect 61% of the positive cases and classed 27% as doubtful, and only 12% could be passed as normal; but, on the other hand, it classed 16% of the negative cases as positive and 22% as doubtful.

The centrifuge deposit test could detect 55% of the positive cases and classed 23% as doubtful, while classifying only 3% as false positives. This

test gave 25% false doubtfuls. The catalase test was useless as it detected less than half of the positive cases, and gave many false positives and false doubtfuls.

The chloride test (digestion method) could detect 57% of the positive cases and gave no false positives. The casein number method detected 91% of the positive cases and gave 8% false positives.

The solids-not-fat in the fat-free milk is a good test for mastitis (solids-not-fat in fat-free-milk lower than 8.76%) 88% of the low solids-not-fat milks examined were found to be infected. "Physiologically low solids-not-fat milks" were thus found to be comparatively rare.

**M. "The diagnosis of *Str. agalactiae* in a liquid selective medium"—S. J. EDWARDS.**

A highly selective medium having the following composition: Lemco broth (pH 7) 1,000 ml., dextrose 5 g., crystal violet (purified) 0.1%, 1 ml., sodium azide 0.1 g., has been found to give good results. It is recommended for checking the clean section of a herd after a blood agar plate diagnosis has been carried out.

**N. "Further studies of the aerobic spore-forming bacilli"—T. GIBSON AND L. E. TOPPING.**

A provisional key to assist in the identification of the commonest species of aerobic spore-forming bacilli is presented. Barritt's modification of the Voges-Proskauer test and the production of acid or acid and gas from glucose are the main diagnostic criteria utilized. The key does not include obligate thermophiles or a few relatively uncommon organisms.

**O. "The production of lactic acid from substances other than sugar by *L. casei*"—C. C. THIEL.**

A strain of *L. casei* has been found to produce more lactic acid in pancreatic and peptic casein and milk digests than is accounted for by the sugar utilized. Also, in pancreatic and peptic casein digests to which no sugar is added, a considerable production of lactic acid (from 0.10 to 0.28 g. per 100 ml.) occurs. A casein suspension does not support growth; added alanine does not increase the lactic acid production in the digests.

Discrepancies between sugar utilized and acid produced do not occur in separated milk and separated milk plus yeast.

**P. "The effect of sterilization upon sugars"—J. G. DAVIS AND H. J. ROGERS.**

Autoclaving sugar solutions for 30 minutes at 120° C. is very much more destructive than "momentary autoclaving," which is only slightly more

destructive than the usual intermittent steaming method. Momentary autoclaving, unlike intermittent steaming, can be relied upon to sterilize media in tubes. Differences in fermentation tests due to heating the sugar in, or apart from, the medium do not seem to be significant. As a standard technique momentary autoclaving of all sugar broths at pH 6.6 is recommended.

## BREEDING

**562. Extending the Use of a Proved Sire.** ALLAN K. BROWN, Guernsey Breeders' Journal, 54, 705, October 15, 1938.

Dr. Allan K. Brown, owner of Brown Ranch, Capitola, California, has practiced artificial insemination in his herd since 1937 and he says that the advantages of this method are:

1. Increased number of progeny obtainable from an ageing and proven sire.
2. Relief from overuse, particularly an old bull.
3. Overcoming physical defects, injuries, or disease in both female and male, such as adhesions in the bull, and cervitis in the cow.

The formula for diluting mediums is:

Water 100 CC.  
Glucose 3%  
With a pH of 7.5.

When the records indicate that several cows are due to come in heat within a period of one or two days the cow(s) coming in heat first are held over a day or two until the others come in heat. The first cow(s) of the group may then no longer accept the bull. However, experience has shown that conception will occur with considerable regularity if the cow is artificially inseminated at this time. One of the cows in heat is bred, the semen removed, diluted and a one cubic centimeter portion discharged into the opening of the cervix of the other cows by an inseminating syringe. Eight cows have been successfully bred by holding them over three days.

His results have been very satisfactory, as out of forty-seven cows bred, forty of them conceived the first time. Eighty per cent of the animals in the breeding herd of 125 females were bred to one bull.

L.R.L.

## FEEDS AND FEEDING

**563. Legume and Grass Silages.** O. M. CAMBURN, H. B. ELLENBERGER, J. A. NEWLANDER AND C. H. JONES. Vermont Agricultural Experiment Station Bul. 434, May, 1938.

The data from one season's trials indicate that:

Legumes and grasses may be successfully ensiled either with or without molasses provided their dry matter contents range between 30 and 40 per cent and preferably as near to 35 per cent as may be.

A dry matter content of less than 30 per cent favors putrefactive fermentation, especially if molasses is not added; one of over 40 per cent tends to prevent tight packing in the silo and induces heating and spoilage.

The ensiling of legumes or grasses is not advisable under ordinary farm conditions unless molasses or acid are added, for the reason that it is hard to control the dry matter content of silage.

The addition of two per cent (40 pounds per ton) of molasses to ensiled timothy and of three or four per cent (60 to 80 pounds per ton) to ensiled alfalfa tends to induce fermentation and to decrease the likelihood of spoilage due to the presence of too much or too little moisture. The odor and palatability of such silages are improved when molasses is used and their feeding value may be slightly enhanced.

The minerals, proteins, and total nutrients are better preserved by ensiling than by ordinary haying methods.

Close packing and exclusion of air are essential to the proper preservation of grass silages, especially those somewhat low in moisture due to wilting after cutting or to maturity of the crop.

An addendum report attached to this bulletin sets forth the results secured in an extensive survey of practical operations throughout the Northeast.

O.M.C.

**564. Digestibility of Alfalfa, Timothy, and Soybeans as Silages and as Hays.** J. A. NEWLANDER, H. B. ELLENBERGER, O. M. CAMBURN AND C. H. JONES. Vermont Agr. Exp. Sta. Bul. 430, March, 1938.

Coefficients of digestibility have been determined with dairy cows for 41 lots of silages and hays made from the 1936 crops of alfalfa, timothy and soybeans. Silages were made both with and without the addition of molasses to freshly cut and slightly wilted cuttings. Hays were made by natural sun curing and by artificial drying. The alfalfa and the timothy were cut at two stages of maturity.

On a dry matter basis most of the hays carried less ether extract and ash than the silages and more nitrogen-free extract. The artificially dried hays contained less fiber than the silages and sun cured hays.

The digestion coefficients of the hays, especially those which were artificially dried, were slightly higher in nitrogen-free extract but significantly lower in ether extract, especially in the case of the sun cured hay. Crude fiber and nitrogen-free extract seemed slightly more digestible in the silages to which molasses was added than in those not so treated.

On a dry-matter basis the silages carried slightly more digestible protein than did the hays. The artificially dried hays carried the most total digestible nutrients, followed in order by the molasses silages, the silages without molasses treatment and the sun cured hays, the latter two being about equal.

O.M.C.

- 565. The Mineral Needs of Farm Animals.** Departments of Animal Industry and Dairy Husbandry, OHIO AGR. EXP. STA., Wooster, O. Ohio Exp. Sta. Spec. Circ. 49. 1937.

**The Vitamin Needs of Farm Animals.** *IBID.* Spec. Circ. 52. 1938.

These two companion circulars present in popular style the requirements of livestock for the various minerals and vitamins and make practical recommendations as to how these requirements can be met. W.E.K.

- 566. Nutritional Requirements of Pregnant and Lactating Rats Studied by the Self-selection Method.** CURT. P. RICHTER AND BRUNO BARELARE JR., Henry Phipps Clinic, Johns Hopkins Hospital, Baltimore, Md. *Proc. Amer. Physiol. Soc., Amer. J. Physiol.*, 123, 1, p. 170, July, 1938.

Ten female rats were allowed to select their food ad libitum from the following 11 substances; casein, sucrose, olive oil, sodium chloride, dibasic sodium phosphate, calcium lactate, potassium chloride, dried baker's yeast, cod liver oil, wheat germ oil, and water.

These animals gave birth to normal litters and nursed most of them until they were weaned 25 days later. The changes in appetite for the 11 substances were strikingly similar in the 10 animals. It was observed that the animals voluntarily ingested large amounts of fat; in fact, over half (52.6%) of the calories were furnished by fat. The fat consumption increased during pregnancy and lactation, supplying 65 per cent of the calories at the end of lactation. Protein furnished 22.3 per cent of the calories before mating, 28.1 per cent at the end of gestation, and 23.8 per cent at the end of lactation. The appetite for sodium chloride, dibasic sodium phosphate, and calcium lactate showed striking increases during pregnancy and lactation. The appetite for wheat germ oil and cod liver oil remained essentially the same. The number of calories increased from 45.3 per day before mating to 58.8 at the end of gestation and to 118.3 at the end of lactation.

The fact that the animals grew and reproduced with as great success as on the McCollum diet, in spite of lower solid food and caloric intake, is evidence of the efficiency of the self selection method in the rat. D.L.E.

- 567. The Effect of Different Per Cents of Protein in the Diet in Successive Generations.** JAMES R. SLONAKER, Dept of Physiology, Stanford University, California, *Amer. J. Physiol.* 123, 2, p. 526, August, 1938.

Five groups of rats and their succeeding six generations were fed rations containing the following per cents of protein, fats and carbohydrates respectively: I—10.3, 12.2, 77.5; II—14.2, 14.2, 71.6; III—18.2, 15.9, 65.9; IV—22.2, 17.8, 60.0; V—26.3, 19.7, 54.0.

The animals comprising these successive generations were all kept in the same room and under the same environmental conditions as their ancestors. Although the animals in group I were smallest at birth and grew slower (both sexually and in skeletal growth) than any of the other groups, their life span was unusually long. Group V grew the fastest up to 70 days of age and attained the greatest final length. On the other hand, the greatest prenatal development took place among the rats fed 18.2 per cent protein. They also proved to be the best mothers.

Protein in the diet equal to, or in excess of, 18.2 per cent interfered with reproduction by increasing sterility. Such a diet also tended to shorten the breeding period and increase the size of the kidneys. In other words, the metabolic processes were quite generally speeded up by increasing the percentage of protein in the ration. The amounts of protein in the rations studied in these trials had no effect on the number of young born or on the sex ratio.

D.L.E.

## FOOD VALUE OF DAIRY PRODUCTS

- 568. The Value of Milk in the Diet.** W. E. KRAUSS, Ohio Agr. Exp. Sta., Wooster, O. Ohio Bimonthly Bull. XXIII, No. 194, Sept.-Oct., 1938.

This is a popular discussion of the food value of milk and is suitable for a 12-minute radio talk. The story builds up to make the point that the ultimate goal for milk consumption should be a pint a day for adults and a quart a day for children.

W.E.K.

## ICE CREAM

- 569. The Frozen Food Industry.** IVAN C. MILLER, McGraw-Hill Pub. Co., New York, Ice Cream Trade J., 34, 9, p. 31, Sept., 1938.

Ice cream manufacturers are watching the development of the frosted food industry. Today he is not an important factor in the distribution of frozen foods. There is every reason to expect that in the future an important portion of retail frozen food distribution will be through the channels of the ice cream manufacturers.

W.H.M.

- 570. Manufacture of Sherbets and Ices.** W. H. MARTIN, Kansas State College, Manhattan, Kans. Ice Cream Trade J., 34, 9, p. 12, Sept., 1938.

This article discusses the problems involved in the manufacture of sherbets and ices. Information is presented on sugar content, stabilizer, color, flavor, use of milk products, overrun, and storage. Formulae for sherbets and ices are given.

W.H.M.

- 571. Triple Scoops.** MALCOLM PARKS. *Ice Cream Trade J.*, 34, 8, p. 13, Aug., 1938.

The use of three No. 30 scoops of ice cream or sherbet in cones and other ice cream dishes has been found by many ice cream manufacturers to be an effective means of increased volume of sales. Mr. Parks presents dealers' cost sheet to show the profit possibilities in this type of merchandising and also gives directions for the preparation and sale of the various items made with three scoops of ice cream.

W.H.M.

- 572. Frozen Food.** PAUL C. TRIMBLE. *Ice Cream Trade J.*, 34, 8, p. 8, August, 1938.

Ice cream manufacturers who are considering the possibility of distributing frozen foods will be interested in this article which describes the experience of the Southern Dairies in the frosted foods field in the southern states.

W.H.M.

- 573. Stabilizers and Their Use in Ice Cream.** L. M. LAMPERT, State Dept. of Agriculture, Sacramento, Calif. *Ice Cream Trade J.*, 34, 7, p. 19, July, 1938.

A definition and classification of stabilizers is given, together with a description of the properties of the various products used in the stabilization of ice cream. The author states that no one can object to their use, provided that they meet reasonable requirements for efficiency, wholesomeness and purity.

W.H.M.

- 574. Increased Sanitation in the Dispensing of Ice Cream.** F. W. FABIAN, Michigan State College, East Lansing, Mich. *Ice Cream Trade J.*, 34, 6, p. 20, June, 1938.

Surveys made by Fabian and others of conditions which surround the sale of ice cream indicate the need for ice cream manufacturers to pay more attention to their product after it leaves the plant. He recommends schools of instruction for those serving ice cream. Measures which will reduce the disease hazard in connection with the sale of ice cream are as follows:

1. Require that all scoops or other implements used in dishing ice cream be kept in water flowing at such a rate so as practically to eliminate the possibility of reproduction and growth of bacteria.

2. Require that all the dishes, silverware, and glassware used in serving ice cream be properly washed. These principles hold for either hand or mechanical washing. Water at a temperature of 140° F. to which has been added one per cent of a good detergent is the lowest best practical temperature to use. After the dishes, glasses, and spoons are washed, they should be rinsed in water at a temperature of not less than 170° F.

3. A bacterial standard of 5000 per gram for flavoring syrups and fruits is suggested.

4. The dispenser should be free of contagious disease and should be clean and neat in his personal appearance.

5. The use of individual package goods should be encouraged by all those interested in producing and serving ice cream. This will do away with most of the objectionable sanitary features which one now observes at many serving places.

W.H.M.

**575. The Influence of Temperature Upon the Extent to Which Freezing Occurs in Ice Cream.** W. C. COLE, Div. of Dairy Industry, Univ. of Calif., Davis, Calif. *Ice Cream Trade J.*, 34, 6, p. 15, June, 1938.

The amount of ice formed at different temperatures in ice cream, ice milk, and sherbets was measured by using a dilatometer. Known weights of the samples to be tested were admitted into the apparatus in such a manner that changes in volume of the sample could be determined. By taking into account the difference in volume before and after freezing at a given temperature and also considering the increase in volume accompanying the change of one gram of water to ice it was possible to calculate the amount of ice formed in the sample at that temperature. The authors summarize their results as follows:

1. Taking into account the changes in volume accompanying the crystallization of ice from solutions it has been possible to measure with a dilatometer the percentages of ice formed in ice cream and related products from 25° C. (13° F.), or slightly lower, to temperatures corresponding to the freezing points of the various samples studied.

2. The results obtained by this method show that at corresponding temperatures the percentages of water frozen in ice milk and ice cream are essentially the same for samples included in this study. Considerable variation occurred, however, in the percentages of ice in these samples depending upon the proportion of water they contained.

3. The results obtained for ices, varied considerably from those obtained for ice cream at corresponding temperatures, both as to the percentages of water frozen and the percentages of ice in the samples.

4. Presumably the data presented can be used as an indication of the extent to which freezing occurs in the commercial manufacture of ice cream and related products.

W.H.M.

## MILK

Abstracts of interest are 561, 568, 576 and 577.

## MISCELLANEOUS

- 576. Safety Improvement Thru Drivers Award System.** EDGAR G. QUESNEL, the Borden Company, *Ice Cream Trade J.*, 34, 9, 13, Sept., 1938.

The Borden Company has experienced a 25 per cent improvement in the safe operation of their vehicles during the past five years by using an award system for their drivers. The awards are usually lapel emblems, badges or certificates and sometimes cash. Accomplishments which may be expected by everyone participating in an Award System of this kind are always worthwhile. They result in good industrial and public relations, better transportation service, lower costs, better work, fewer interruptions, few accidents, less personal injury and property damage, minimum of public liability claims, and most important of all the conservation of the most valuable asset the company has—the life, happiness, ambitions and future accomplishments in the field of constructive service—the employee himself.

W.H.M.

- 577. Cold Storage Lockers.** P. EDWIN THOMAS. *Ice Cream Trade J.*, 34, 7, p. 21, July, 1938.

“Practically unknown five years ago, some 2,000 cold storage locker businesses are reported in operation in almost half the States of the Union, with an investment of nearly \$25,000,000.” A floor plan for a 320 locker plant 35 feet wide by 70 feet long is illustrated. The cost of such a plant is estimated at \$25 to \$50 per locker and according to the figures presented, it would be possible for the plant to yield a return on investment of 10 per cent.

W.H.M.

- 578. Index of Research Projects, Works Progress Administration.**

The results of some 2,000 research projects carried on as part of the federal work relief program are summarized briefly in a digest and index which has been published by the Works Progress Administration. This volume of 291 pages contains a concise statement of the principal conclusions of each study and an alphabetical subject index to the contents. The reports on these projects touch upon nearly every field of natural and social science and many of them have appeared in the form of articles in scholarly journals. However, several hundred of the reports summarized in this index are in manuscript form, and arrangements have been made with the American Documentation Institute whereby microfilm copies of the original reports will be furnished at nominal rates for the use of research specialists. A small edition of this volume has been prepared for distribution to the larger public and university libraries, where it will be available for reference, and for government departments, industrial concerns and research

foundations. A limited supply of copies of this Index of Research Projects are still available. Requests should be addressed to the Works Progress Administration in Washington. H.K.H.

## PHYSIOLOGY

- 579. Concerning the Metabolism of Fat and Carbohydrate.** JOSEPH L. DONNELLY, Fort Thomas, Ky. *Amer. J. Physiol.* 124, 1, p. 126, October, 1938.

From a selected group of data published by other workers, the author draws some very important inferences on the relation of total acidity of the body to fat and carbohydrate metabolism. He points out that as a general mode of behavior there is an increase in the size of the globules of fat in the tissues with any increase in the acidity of the animal. This increase in size of fat globules results in a loss of reactivity which parallels the decrease in dispersion of the fat. Moreover, the activity of lipase and bile is diminished by acid. After pointing out the apparent relationship between the concentration of sugar and fat in the blood, the author draws the conclusion that "the size of the tissue fat globule and the concentration of fat and carbohydrate in the blood vary directly while the utilization of fat and carbohydrate vary inversely with the total acidity of an animal."

The statement of Professor Denning (*Research and Progress* 4: 41. 1938) that soya bean meal induces alkalosis is of interest here. D.L.E.

- 580. The Effect of Pregnancy and Lactation on Growth in the Rat.** II. H. COLE AND G. H. HART, College of Agriculture, University of California, Davis. *Amer. J. Physiol.* 123, 3, p. 589, September, 1938.

The authors show that pregnancy stimulates skeletal and tissue growth in the rat beyond that found in non-bred littermate controls. The excess growth during pregnancy is accompanied by, and presumably dependent upon, an increased food consumption manifested by the second day after conception. It is postulated that the copulatory act brings about a nervous stimulation of the anterior pituitary, resulting in the secretion of one or more hormones involved in inducing an increased appetite.

The excess gains made by the pregnant rats remain fairly constant for the first six pregnancies, after which further pregnancies have less effect. The excess gains are made, for the most part, during pregnancy although rats suckling four to six young continue to gain as rapidly as non-bred controls. D.L.E.

- 581. The Ratio of Arterio-venous Differences of Certain Substances to Quantities Secreted by the Mammary Gland.** J. C. SHAW AND

W. E. PETERSEN, Department of Dairy Husbandry, University of Minnesota, Minneapolis, Minn. Proc. Amer. Physiol. Soc., Amer. J. Physiol. 123, 1, p. 183, July, 1938.

Arterio-venous differences were determined on bloods drawn from the internal iliac and external pudic arteries and the subcutaneous abdominal milk veins for calcium, glucose, lactic acid, fat and amino acids.

The ratio of volume of blood flow traversing the gland to the amount of milk secreted was 1:387 when calculated from the blood calcium differences and the calcium in the milk. The ratio of the combined glucose and lactic acid differences to the lactose in the milk was 1:390 which is in good agreement with the calcium ratio. The differences in neutral fat indicated a ratio of 344 volumes of blood per volume of milk. The amount of amino acids removed by the gland are inadequate to account for more than 40 per cent of the proteins of the milk on this basis. The essential data follow.

	# of analyses	Arterial venous differences	Per cent in milk	Ratio
Amino-acid nitrogen	15	0.46 mgm. %	500 (total nit.)	1:1087
Glucose-lactic acid	17	12.44 "	4860 (Lactose)	1:391
Fat	39	11.63 "	4000	1:344
Calcium . . . . .	20	0.31 "	120	1:387

D.L.E.



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